Off-target effects of RAAS-inhibition: importance on renal outcomes in patients with diabetes

CIP-GEGEVENS KONNIKLIJKE BILBLIOTHEEK, DEN HAAG

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Proefschrift Groningen met literatuur opgave en samenvatting in het Netherlands

ISBN: 978-90-367-5457-6

This work was performed within the framework of the research school GUIDE (Groningen University Institute for Drug Exploration).

Financial support was kindly provided by University of Groningen, University Medical Center Groningen.

Cover design and page layout: Shangguan Design, Beijing, China and Yan Miao
Printed by: Wohrmann Print service, Zutphen, The Netherlands

RIJKSUNIVERSITEIT GRONINGEN

Off-target effects of RAAS-inhibition: importance on renal outcomes in patients with diabetes

Proefschrift

ter verkrijging van het doctoraat in de
Medische Wetenschappen
aan de Rijksuniversiteit Groningen
op gezag van de
Rector Magnificus, dr. E. Sterken,
in het openbaar te verdedigen op
maandag 21 mei 2012
om 11:00 uur

door

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geboren op 2 februari 1981

te Tianijn, China

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CHAPTER 1

Introduction and aims of the thesis

Prevalence of type 2 diabetes and its associated renal complications

Over the past decades it has been obvious that the prevalence of type 2 diabetes is increasing rapidly.(1) The World Health Organization reports that 346 million people suffer from diabetes and estimates indicate that the number of people with diabetes will double in 2030. Type 2 diabetes significantly contributes to morbidity, premature mortality and places a large burden on national health care budgets.

Patients with type 2 diabetes frequently present renal problems starting with sign of hyperfiltration followed by microalbuminuria (>30 mg of albumin in the urine per 24hr). The presence of microalbuminuria may be present in approximately 20 to 30% of all patients with type 2 diabetes at the time of diagnosis. The progression to further involvement in the kidneys is shown when urinary albumin excretion rises to macroalbuminuria (> 300 mg/24hr) which occurs annually in approximately 3% of the microalbuminuric patients. Patients with macroalbuminuria are at risk for progressive renal function decline and loss of hormonal functions that are regulated by the kidneys such as vitamin D metabolism and erythropoietin production. Between 10 and 20% of all people with diabetes will ultimately require dialysis or transplantation if they did not prematurely die due to cardiovascular diseases. Given the high prevalence of diabetes and nephropathy in combination with the poor prognosis of these patients it is essential to optimize the use of currently available therapies as well as finding novel therapies.

Effects of Angiotensin Receptor Blockers in patients with type 2 diabetes and nephropathy

Blood pressure lowering unambiguously contributes to reduce the risk of progressive renal function loss in patients with diabetes. During the last decades a vivacious debate has been ongoing on the topic which antihypertensive drugs to use to halt the progressive loss of renal function. Already in 1992 Bjorck et al showed that agents intervening in the renin-angiotensin-aldosterone-system (RAAS) more effectively reduce the risk of nephropathy than B-blockade.(2) Two years later the Angiotensin Converting Enzyme Inhibitor (ACEI) captopril was shown to reduce the risk of doubling of serum creatinine or End Stage Renal Disease (ESRD) in type 1 diabetic patients.(3) These early trials provided the first evidence of the clear beneficial effects of RAAS blockade. The introduction of Angiotensin Receptor Blockers (ARBs), which inhibit the activity of the RAAS by blocking the Angiotensin Type 1 Receptor, provided the possibility to further study the renoprotective effects of RAAS-inhibition in type 2 diabetic patients. The ROADMAP and IRMA-2 trials have shown the value of ARBs in early stages of renal disease and demonstrated that olmesartan or irbesartan delayed the onset of micro- and macroalbuminuria, respectively.(4, 5) The

RENAAL and IDNT trials demonstrated that ARB treatment (losartan or irbesartan) in patients with type 2 diabetes and established nephropathy reduced the incidence of doubling serum creatinine concentration and ESRD.(6, 7)

The superior effects of RAAS-blockade with ACEIs or ARBs appeared to be related to their albuminuria lowering effects. High albuminuria has consistently been demonstrated to be one of the most important risk markers for ESRD in a range of patients and settings. Intervention in the RAAS with ACEi or ARB reduces albuminuria by approximately 30 to 40%.(8) The reduction in albuminuria obtained in the first months of therapy appears to be related with the progression of renal disease in the subsequent years. This observation was initially found in type 2 diabetic and non-diabetic patients.(9, 10) Detailed post-hoc analyses from large scale clinical trials subsequently confirmed these findings in larger international cohorts and showed that the reduction in albuminuria in the first 6 months is linearly related with the risk reduction for ESRD. Interestingly, the reduction in albuminuria during ARB treatment, rather than the reduction in blood pressure, appeared to be the driving determinant for renoprotection in these trials.(11, 12) Thus, although ARBs are registered as antihypertensive drugs they appear to have off-target effects, such as albuminuria lowering, that contribute to their beneficial effects on clinical meaningful endpoints. This means that one should not only look at the on-target blood pressure effect to estimate the renoprotective effect, but also take the off-target anti-albuminuric effects into account.

Multiple off-target effects of Angiotensin Receptor Blockers

Apart from the blood pressure and albuminuria lowering effects of ARBs, these drugs have more off-target effects on renal risk markers. Blocking the RAAS has been shown to increase serum potassium as a result of decreased aldosterone activity.(13) Furthermore, it is known that some ARBs decrease serum uric acid.(14) Finally, it is known that RAAS-inhibition reduces hemoglobin levels possibly due to inhibition of erythropoiesis.(15) Since serum potassium, uric acid, and hemoglobin are independent risk markers for renal disease,(16, 17) ARB induced changes in these renal risk markers may influence the ultimate effect of ARBs on hard renal outcomes.

Drug registration and development

To register a new drug in renal and cardiovascular disease the drug must be proven to be effective and safe. To prove the drug's efficacy and safety, the on-target effects on the parameter of interest (e.g. blood pressure for antihypertensive drug or glucose for oral glucose lowering drug) are determined and used to estimate the potential long-term renal protection. To verify the estimated

long-term renal protection, the short-term evaluation is followed by a hard-outcome study looking at meaningful outcomes such as dialysis or renal transplantation. The latter thus requires large and expensive randomized controlled clinical trials with long-term follow-up. Several recent examples in renal and cardiovascular disease illustrate the failure of this process. Rosiglitazone was registered in Europe in 2000 based on its beneficial effect on HbA1c. However, meta-analyses have shown that, despite the HbA1c lowering effect, the drug increases cardiovascular risk leading to marketing suspension in 2010.(18, 19) In addition, the combination of an ACEi and ARB more effectively lowered blood pressure than the single use of these agents but increased renal risk in the ONTARGET trial.(20) These examples, and many others exist, indicate that the drugs did not deliver what they were intended to do estimated from their effects on the parameters of interest. Thus, only evaluating drug effects on the on-target risk factor is insufficient to estimate the ultimate drug effect on hard outcomes. It is likely that establishing the integrated effect of a drug on the on-target and off-target risk markers will result in a better prediction of drug effects on hard renal outcomes. Such an approach has obviously major consequences for drug benefit-risk assessment, cost of drug development, drug innovation and for society.

Aim of the thesis

To assess off-target effects of Angiotensin Receptor Blockers on renal risk markers in patients with type 2 diabetes and nephropathy and the relationship between off-target drug responses and effects on hard renal outcomes. This should ultimately result in the construction and validation of a multiple parameter risk response score based on on-target and off-target effects of ARBs in order to better estimate long-term drug efficacy.

Chapter 2 reviewed the off-target effects of various drugs in patients with type 2 diabetes. This review focused on the relationship between the off-target effects of various drugs on novel risk markers and their relationship with renal and cardiovascular outcome in patients with diabetes. In **chapter 3** the effect of losartan was assessed on a well-known but emerging risk marker in the renal arena, namely serum uric acid. In this study the magnitude of the reduction in serum uric acid induced by losartan was associated with the degree of renal protection and the contribution of the change in serum uric acid to the renoprotective effect of losartan was calculated. **Chapter 4[a]** describes a similar analysis as described in chapter three and focused on the losartan induced change in serum potassium and its relationship with renal outcome. Importantly, the change in serum potassium induced by a drug is commonly considered a safety issue. However, this chapter

shows that a drug induced increase in serum potassium also affects drug's renal outcome. This implies that one should not only evaluate the effect of a drug on serum potassium within the "safety" context but it should also be considered in drug efficacy evaluation. In **chapter 4[b]** the effects of the ARB losartan on serum potassium is further investigated in individual patients. The general believe is that within a patient the drug response in multiple parameters is concordant. This implies that a reduction in blood pressure is always accompanied by a reduction in albuminuria or an increase in serum potassium. However, recent studies have questioned this dogma and have shown that the effect of a drug on multiple risk markers is not always concordant within a patient. More specifically, ARBs may decrease blood pressure but at the same time increase albuminuria or *vice versa* within a patient. As both the change in blood pressure and the change in albuminuria predict long-term renoprotection, such data support a dual strategy of both targeting and optimizing the blood pressure and albuminuria response. In chapter 4[b] the response to losartan in two off-target risk markers, namely albuminuria and serum potassium is determined in individual patients. The relationship of different response patterns in these off-target risk markers with long-term renoprotection is also reported in this chapter. The chapters in this thesis in combination with existing literature demonstrate that ARBs, registered as antihypertensive drug, influences multiple renal risk markers and most of them influence renal outcome, either positively or negatively. This implies that calculating the integrated effect of an ARB on all these risk markers will likely result in a better prediction of the true renoprotective effect of an ARB on clinical meaningful endpoints, such as dialysis or renal transplantation. In **chapter 5** we constructed and validated a multiple parameter risk response score to determine the integrated effect of a drug, in our case an ARB, on on-target and off-target risk markers in order to accurately predict the renoprotective effect of ARBs.

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CHAPTER 2

Drug-induced changes in risk/biomarkers and their relationship with renal and cardiovascular long-term outcome in patients with diabetes

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Clinical Chemistry 57:2 186-195 (2011).

Abstract

Background: Optimal renal and cardiovascular risk management in diabetic patients includes optimal maintenance of blood pressure, glucose and lipid control. Although optimal control of these risk factors or "risk/biomarkers" has proven efficacy, it often is difficult to achieve. Consequently, the risk for renal and cardiovascular complications remains devastatingly high. Many risk/biomarkers have been discovered that accurately predict long-term renal and cardiovascular outcome. However, the aim of measuring risk/biomarkers may not be only to determine an individual's risk, but also to use the risk/biomarker level to guide therapy and thereby improve long-term clinical outcome.

Content: This review describes the effects of various drugs on novel risk/biomarkers and the relationship between (drug induced) short-term changes in risk/biomarkers and long-term renal and cardiovascular outcome in patients with diabetes.

Summary: In post-hoc analyses of large trials, the short-term reductions in albuminuria, transforming growth factor-β and N-terminal pro-B-type natriuretic peptide (NT-proBNP) induced by inhibitors of the renin-angiotensin-aldosterone system were associated with a decreased likelihood of renal and cardiovascular outcomes in the long-term. However, the few studies that systematically investigated the utility of prospectively targeting novel risk/biomarkers such as hemoglobin or NT-proBNP failed to demonstrate long-term cardiovascular protection. The latter examples suggest that although a risk/biomarker may have superior prognostic ability, therapeutically changing such a risk/biomarker does not necessarily improve long-term outcome. Thus, to establish the clinical utility of other novel risk/biomarkers, clinical trials need to prospectively examine the effects of therapeutically-induced changes in single or multiple risk/biomarkers on long-term risk management of patients with diabetes.

Introduction

Patients with diabetes mellitus are prone to develop a broad range of complications. The most common of these are renal and cardiovascular (CV) complications that are associated with a large burden of social dysfunction and with high risk of premature death.

Several modifiable risk factors are associated with poor renal and CV outcome, including blood pressure, plasma glucose and lipid concentrations, smoking, and body weight. This review will distinguish between modifiable risk factors and biomarkers in the following way: A modifiable risk factor or risk marker (hereinafter called risk factor or risk marker) is a biological characteristic that is causally correlated to a clinical endpoint, and its intervention-induced change should predict outcome; the risk factor differs from a biomarker in that the latter is a biological characteristic indicating a normal biologic process, a pathogenic process or an effect of treatment on such a process.(1) Biomarkers are often used as surrogate endpoints in clinical studies. In such studies the biomarker is used to substitute for a clinical endpoint. It is hoped that the biomarker will directly reflect the disease process under investigation but could be indirectly related. It is therefore possible that changes in the biomarker will not directly correlate to the treatment or desired outcome.

Although all risk markers can be considered biomarkers, it is likely that only a subset of biomarkers will achieve risk marker status. Blood pressure is a clear example of a risk marker because it is causally related to CV disease and the reduction in blood pressure induced by an antihypertensive agent is related to the degree of CV risk reduction.(2) Angiotensin is an example of a biomarker, where high angiotensin concentrations are a reflection of renal disease and where therapy induced change in Angiotensin concentrations may reflect the efficacy of the therapy, without a direct causal relationship between Angiotensin and renal disease. There are also examples where the boundaries between risk marker and biomarker are overlapping. For example, albuminuria is a reflection of renal damage. As such it is a biomarker of renal disease state. On the other hand, albuminuria is also believed to be a causal factor in progressive renal function loss. Treatments that lower albuminuria lower the risk for renal and cardiovascular disease. In this respect albuminuria is also a risk factor/marker. Thus the differentiation between risk marker and biomarker is not always as well-defined as one may infer. Throughout this review we will use the term risk/biomarker without taking away the potential differential relation.

In clinical practice there are several risk/biomarkers that can be used as a target to improve renal and CV protection. However, it appears that optimal control of these risk/biomarkers is difficult to achieve. This is illustrated by the multi-factorial intervention trial Steno-2. In this study only a small proportion of patients achieved optimal risk/biomarker control despite intensive renal

and CV protective therapy.(3) Consequently, a substantial proportion of patients remain at a high renal and CV risk.(4)

A host of articles on novel risk/biomarkers is currently being published in medical literature. Many of these publications intend to show that the novel risk/biomarkers at hand has the ability to identify more accurately patients with diabetes at risk for development of renal and CV diseases. (5, 6) Nevertheless, although this may be true, one has to realize that the goal of measuring risk/biomarkers is not only to determine an individual's risk but also to use the risk assessment to guide appropriate therapy and thereby to improve long-term clinical outcome. It is therefore important to obtain insight into the effects of therapeutic approaches on short-term changes (observed within the first months after initiation of therapy) in these new risk/biomarkers, and to delineate whether these short-term drug-induced risk/biomarker changes are associated with long-term reductions in risk for renal and CV outcomes in ensuing years. Such information will allow the doctor and patient to use the risk/biomarker as a risk estimator as well as use it as an estimate for therapy success. This review will discuss (1) the impact of treatment on novel renal and CV risk/biomarkers (excluding traditional risk factors such as blood pressure, glucose, lipids, body weight, and smoking), and (2) delineate whether short-term treatment-induced changes in single or a panel of multiple risk/biomarkers predict changes in risk for long-term renal and CV outcomes.

Targeting single risk/biomarkers

Albuminuria

Albuminuria, a marker of generalized vascular dysfunction, is one of the most frequently evaluated risk/biomarkers in patients with diabetes. Large observational studies in patients with diabetes have shown that it is a valuable marker in predicting the risk for renal and CV disease.(7-9) In addition, various drugs are known to lower albuminuria. Well known are the albuminuria-lowering effects of renin-angiotensin-aldosterone system (RAAS) inhibitors. These agents lower albuminuria by approximately 40%.(10) The (short-term) reduction in albuminuria achieved with RAAS inhibitors may be a critical step in achieving long-term protection against renal events (defined throughout this review as the need for chronic dialysis or renal transplantation) and CV events. Post-hoc analyses from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan Study (RENAAL) trial in patients with diabetes illustrated that each 50% reduction in albuminuria induced by treatment with an angiotensin receptor blocker (ARB) during the first months of therapy was associated with 45% and 18% risk reduction for renal and CV events during the ensuing 3.4 years follow-up respectively (Table 1 and Figure 1).(11, 12) Similar results were observed in the

Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) trial. The ADVANCE trial illustrated that each halving of albuminuria during follow-up, achieved with combination therapy consisting of an Angiotensin Converting Enzyme inhibitor (ACEi) and diuretic, resulted in 20% risk reduction for CV events.(7)

A relevant scientific question is whether the short-term albuminuria lowering effects of RAAS inhibitors, registered as antihypertensive drugs, are mediated through their effect on blood pressure only, or whether they are the result of a combination of effects (including the lowering of blood pressure). Indeed, RAAS inhibitors have multiple other effects such as lowering of albuminuria. If albuminuria reduction confers renal and CV protection independent of changes in blood pressure, or other risk/biomarkers, such evidence would support the validity of albuminuria as an independent target for renal and CV protective therapy. The renoprotective effects of RAAS inhibitors beyond blood pressure control were initially discovered in non-diabetic patients. The Ramipril Efficacy in Nephropathy (REIN) trial showed that ramipril lowered the risk of End Stage Renal Disease as compared to conventional antihypertensive therapy at similar level of blood pressure control.(13) These results extended to the type 2 diabetic population. The Irbesartan Diabetic Nephropathy Trial compared the effects of an ARB (irbesartan), Calcium Channel Blocker (amlodipine), and placebo in patients with diabetic nephropathy.(14) The rationale to include a calcium channel blocker arm in this trial was to determine blood pressure independent renoprotective effects of the ARB.(15) The trial showed that irbesartan significantly lowered the risk for renal events compared to amlodipine despite similar blood pressure control. A post-hoc analysis of this trial further illustrated that irbesartan's superior renoprotective effect could be in large part attributed to its effect on proteinuria reduction.(16) However, when the authors compared the renoprotective effects of irbesartan versus amlodipine at similar degrees of blood pressure and proteinuria reduction, irbesartan still provided better renoprotection. This finding indicated that the pharmacological effects of irbesartan could not be fully explained by its effects on blood pressure and proteinuria alone and implied that irbesartan's effect on other, yet unidentified, risk/biomarkers, was involved in its long-term renoprotective effect. The results of this study argued for the simultaneous measurement of short-term changes in multiple risk/biomarkers in explaining the overall pharmacological effects of an agent on long-term hard renal and CV outcomes.

Further indirect evidence supporting the validity of albuminuria as an independent target for renal and CV protective therapy comes from a detailed analysis by Eijkelkamp et.al.(17) In this analysis of the RENAAL trial the blood pressure response to an ARB (losartan) was dissociated from the albuminuria response. The study showed that long-term renoprotection was related to the

degree of albuminuria lowering and to a lesser extent to the degree of blood pressure lowering. Thus, RAAS inhibitors play a unique role in renal and CV therapy because of the protection they afford which is mediated, at least in part, through their effect on albuminuria.

Although of interest, these post-hoc analyses can only be interpreted as hypothesis generating. To evaluate monitoring and targeting albuminuria as an effective treatment strategy, one group of patients should be assigned to frequent measurement and adjustment of medication if targets are not met, while the other group receives standard care. Such a design would isolate the role of targeting albuminuria by focusing on the additive effect of monitoring albuminuria as compared with standard therapy, and provide a better approach to establish the clinical relevance of targeting albuminuria for renal and CV protection. Such a trial has not yet been conducted, although Hou et.al. came very close with the design and results of the Renoprotection of Optimal Antiproteinuric Doses (ROAD) trial, conducted in non-diabetic patients. Hou et al. aimed to specifically target albuminuria using dosages of ACEis or ARBs well above the dosage that is conventionally used for blood pressure reduction. It is known that such high dosages of ACEis or ARBs confer additional antiproteinuric effects beyond their blood pressure lowering effect.(18, 19) Hou et.al. reported that targeting albuminuria with optimal antiproteinuric dosages of ACEis or ARBs resulted in much better renal protection than conventional antihypertensive therapy despite similar blood pressure control.(20) Although these results are promising, further studies are needed to resolve the issue whether specific lowering of albuminuria results in renal and CV protection.

Since RAAS inhibition forms the mainstay therapy for renal and CV protective therapy, the albuminuria lowering effects of novel agents are now tested on top of RAAS inhibition. Thiazolidinediones, oral glucose lowering drugs, act through stimulation of the peroxisome proliferator activated receptor γ. These drugs have been shown to significantly lower albuminuria in patients with diabetes.(21, 22) Another target has been the Vitamin D receptor. Studies have indicated that Vitamin D Receptor Activators (VDRA) exert albuminuria lowering effects through suppression of the RAAS and anti-inflammatory effects.(23, 24) Apart from VDRA therapy, HMG-CoA reductase inhibitors (statins) also lowered albuminuria, but this seems to be a specific drug effect since not all statins uniformly lowered albuminuria.(25, 26) Another target to lower albuminuria has been blocking the endothelin type A receptor in the endothelin system, which seems to play a role in the pathogenesis of albuminuria. Several studies have shown that blocking the endothelin type A receptor significantly reduced albuminuria up to 40% in patients with type 2 diabetes and nephropathy beyond optimal RAAS blockade.(27, 28) Although effective, the side effects of endothelin antagonists, in particularly fluid overload, have been a cause of concern and

may blunt the CV protective effects of albuminuria lowering. To date, no hard endpoint trial has been completed with either VDRAs, statins, or endothelin antagonists. Thus, whether short-term reductions in albuminuria during either VDRA, statin, or endothelin antagonist therapy relate to reductions in the risk for hard renal and CV events remain unknown.

The utility of (changes in) albuminuria as a risk/biomarker for renal and CV disease have been debated extensively. Critics have focused on at least three issues: variability of the albuminuria within an individual, absence of albuminuria in patients with renal or CV function loss, and finally large trials that deny the importance of albuminuria as a predictor or a target for treatment. First, studies have shown that the variability in albuminuria is large and as such albuminuria would not be a good risk/biomarker. Indeed, large random day-to-day fluctuations in any risk/biomarker hamper the accuracy and precision to predict changes in renal and CV risk. When examined at the individual level, studies have shown that albuminuria varies from day-to-day.(29) However, when examined at the group level, the variability in albuminuria was equal to the variability in other risk/biomarkers.(27) Secondly, studies have shown that patients without albuminira can have progressive renal function loss. This is no surprise, since albuminuria is just like other risk/biomarkers, one of the many contributors to renal and CV disease. Obviously, other factors likely play a role in disease progression.(30) Importantly, the available evidence clearly has shown that increased levels of urine albumin when present are an excellent predictor of later renal and cardiovascular problems in patients with and without diabetes.(31) Thirdly, the Ongoing Telmisartan Alone and in combination with Ramipril Global Endpoint (ONTARGET) trial showed that despite additional albuminuria reduction, combination therapy of ACEi and ARB did not confer CV protection and even increased the risk of renal disease.(32, 33) This led to lively discussion about the validity of albuminuria as a risk/biomarker of renal disease and recommendations to dismiss albuminuria as a surrogate endpoint for renal and cardiovascular protection. Intriguingly, the discussion focused on albuminuria, although blood pressure was also further reduced in the combination arm. Since the combination arm showed no further protection in long-term outcomes, it would have been equally valid to consider dismissing blood pressure as a valid risk/biomarker! With regard to albuminuria in the ONTARGET trial, a recent analysis provided ample evidence that both baseline albuminuria as well as changes in albuminuria during the first years predicted the risk for renal and CV events in the following years.(34) This was in contrast with the earlier ONTARGET renal report which gave the impression that albuminuria was not a valid renal risk predictor. Thus, the recent ONTARGET analysis further substantiates numerous previous studies demonstrating that in individual patients changes in albuminuria are an excellent predictor for changes in future renal and CV risk.

Figure 1: Associations between the proportional change in different renal and cardiovascular (CV) risk/biomarkers and the risk for CV outcomes. The data show the relationship between the short-term change in respectively albuminuria (RENAAL) (11), proBNP (Val-HeFT) (57) and hs-CRP (JUPITER)(56) and CV outcome. The JUPITER trial reported CV hazard ratio for rosuvastatin assigned patients with less than 50% and more than 50% reduction in hs-CRP compared to placebo which was used as reference. For illustrative purposes, it was assumed that a reduction in hs-CRP of less than 50% resulted in a mean reduction of 30% whilst a reduction of more than 50% resulted in a mean reduction of 80%.

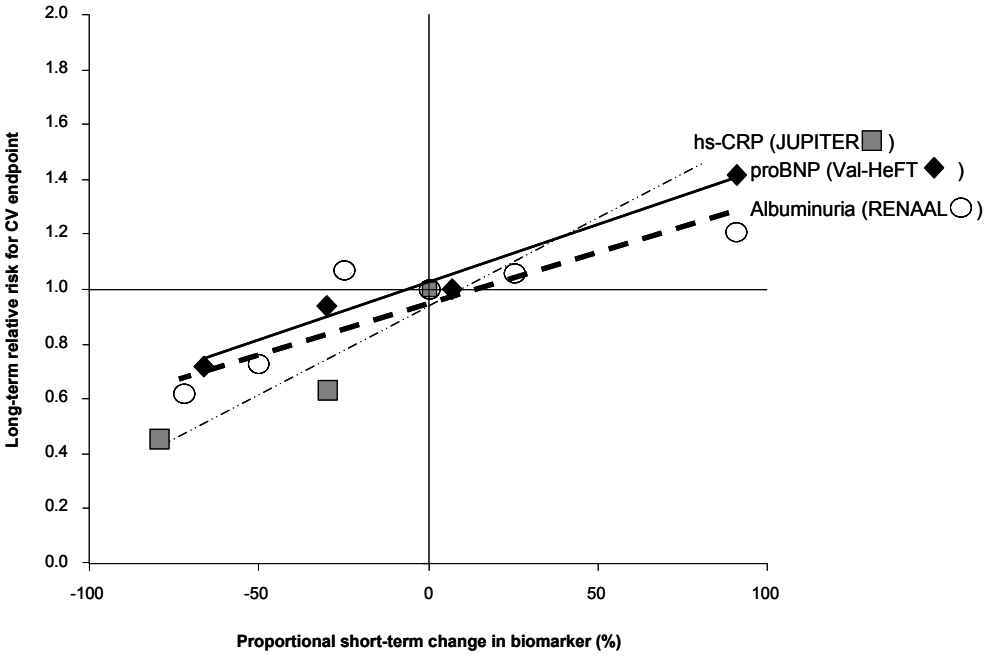

Table 1: Overview of studies in patients with diabetes determining the association between drug induced changes in risk/biomarkers with long-term renal or CV risk change.[a]

Risk/biomarker	Trial	Intervention	Risk/biomarker change	Change of long-term renal or CV risk
Single risk/biomarkers				
Albuminuria	RENAAL	Losartan 100 mg/ day vs. placebo	Compared to placebo, albuminuria was reduced with 32% in albuminuria after 6 months.	Each halving of albuminuria during the first 6 months was associated with a reduction in the risk of renal and CV disease of respectively 45% and 18% during 3.4 years follow-up.
	IDNT	Irbesartan 300 mg/ day vs. Amlodipine 10 mg/day vs. placebo	Compared to placebo, albuminuria levels were 30% lower in irbesartan treated subjects after 12 months.	Each halving of proteinuria during the first 12 months was associated with a risk reduction in end stage renal disease of 56% during 2.9 years follow-up.
TGF-β	Captopril Trial	Captopril 75 mg/ day vs. placebo	Captopril caused a 25% reduction in TGF- β compared to placebo during the initial 6 months.	An inverse correlation was found between the change in TGF-β and the percentage change in eGFR over the ensuing 2 years (r=-0.45; P=0.008).
Hemoglobin	TREAT	Darbepoetin-α vs. placebo	Hemoglobin levels increased from 104 to 106 g/L and from 104 to 125 g/L in respectively placebo and darbepoetin-α treated subjects.	Darbepoetin-α increased hemoglobin levels but it did not reduce the risk of renal or CV events. A post-hoc analysis showed that despite receiving high dosages of Darbepoetin-α, patients with a poor response during the first 4 weeks had a higher risk of CV outcomes.
CRP	JUPITER	Rosuvastatin 20 mg/day vs. placebo	3573 (46%) participants assigned to rosuvastatin had a reduction in hs-CRP more than 50%.	Compared to placebo, participants assigned to rosuvastatin who achieved a CRP reduction more than 50% had a 54% CV risk reduction.
NT-proBNP	Steno-2	Intensive vs. conventional multifactorial intervention	Compared to placebo, NT-proBNP levels were 6.5 ng/ L lower in intensive treatment arm after 2 years.	A 10 ng/L reduction in NT-proBNP during the first 2 years was associated with a significant 1% CV risk reduction during a median follow-up of 7.8 years.
Multiple risk/biomarkers				
	Steno-2	Intensive vs. conventional multifactorial intervention	Compared to conventional intervention, intensive treatment reduced LDL cholesterol 0.3 mmol/L, HbA1c 1.0%, albuminuria 32 mg/24hr, and systolic blood pressure 4 mmHg.	Intensive treatment attenuated the risk of nephropathy (risk reduction 44%) retinopathy (risk reduction 55%), CV events (risk reduction 59%) and death (risk reduction 46%).

[a]All listed studies enrolled patients with diabetes except the JUPITER trial which included apparently healthy individuals with high CRP.

Abbreviations: BNP Brain Natriuretic Peptide; CRP, C-reactive Protein; JUPITER, Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin, IDNT, Irbesartan in Diabetic Nephropathy; RENAAL, Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan; TREAT, Trial to Reduce Cardiovascular Events with Aranesp Therapy.

Transforming Growth Factor-β

Transforming Growth Factor-β (TGF-β) has a key role in the processes that lead to an increase in matrix components, infiltration of macrophages in renal tissue, and loss of nephrons eventually leading to diabetic nephropathy. Therefore, it is not surprising that presence of TGF-β has been shown to predict the onset of End Stage Renal Disease.

TGF-β is an important transducer of the pathogenetic effects of Angiotensin II and its levels are controlled by the RAAS. This has led to studies investigating whether the effects of RAAS-inhibitors on TGF-β could account for the renoprotective effects of RAAS-inhibitors beyond blood pressure and albuminuria lowering. To this end, the changes in serum TGF-β levels at 6 months induced by the ACEi captopril, were measured and correlated to the 2-year rate of renal function decline in patients with type 1 diabetes.(35) Captopril caused a significant decline in TGF-β levels compared to placebo. The degree of TGF-β reduction coincided with the degree of glomerular filtration rate (GFR) preservation during the ensuing 2 years follow-up (table 1). Thus, if TGF-β declined at 6 months the rate of renal function decline during the subsequent 2 years follow-up was smaller. An important question was whether the changes in TGF-β were independent of changes in albuminuria. Another study provided more insight into the independent effects of RAAS-inhibition on TGF-β levels. Agarwal et al. found that 4-weeks treatment with the combination of an ACEi and an ARB significantly attenuated TGF-β levels. Interestingly, the reductions in TGF-β levels occurred independent of changes in 24-hr urinary protein excretion or blood pressure.(36) These data suggest that the effect of RAAS-inhibition on TGF-β may in part explain its renal protective effect. However, the definitive answer on whether TGF-β suppression is independently associated with renoprotection should come from randomized controlled trials.

Hemoglobin

Anemia is a common finding in patients with diabetes and it has potential to negatively affect well-being and social functioning. Clear evidence is available that anemia is an independent potent risk factor for CV disease.(37-39) The role of anemia as CV risk factor appears to extend to progression of chronic kidney disease. In patients with type 2 diabetes, anemia has been documented to be an independent risk factor for doubling of serum creatinine (50% reduction in Glomerular Filtration Rate) or End Stage Renal Disease.(9)

Various drugs affect hemoglobin levels. First, ARBs are known to lower hemoglobin levels (an unwanted side effect). The reductions in hemoglobin during ARB therapy appear not to affect the overall efficacy of ARBs on the progression of renal disease. Toto et.al. demonstrated that the

long-term renoprotective effects of the ARB losartan in individuals with diabetes and nephropathy persisted in the presence of a significant reduction in hemoglobin.(40) These data indicated that a reduction in hemoglobin during the initial months after start of ARB therapy did not necessarily imply a dose reduction or discontinuation of treatment altogether.

Erythropoiesis-stimulating-agents (ESA) are a class of agents that are intended to target hemoglobin levels and bring them towards normalcy. Despite the absence of high quality outcome data, ESA therapy has been frequently used based on the expectation that correction of anemia improves renal and CV outcome. Data from the Trial to Reduce Cardiovascular Events with Aranesp Therapy (TREAT), however, created a dilemma: ESA-therapy increased hemoglobin levels after just 3 months therapy but did not confer long-term renal or CV protection.(41) These results were highly remarkable because theoretically increasing oxygen delivery and improving CV hemodynamics should improve vascular outcomes.(42) Although a lot of attention has centered on the hemoglobin targets used in these trials, another explanation could be that high ESA exposure itself might account for the detrimental effects. Indeed, a post-hoc analysis of the TREAT trial demonstrated that patients with a poor hematopoietic response to ESA therapy (those within the lowest quartile of the 4-weeks hemoglobin change) were more likely to experience a CV event or die as compared to responsive patients (Table 1)(43). These data underscore the importance of considering individualized therapeutic responsiveness and limiting dose escalation in those not attaining targeted hemoglobin goals. Further studies, such as the Clinical Evaluation of the DOSe of Erythropoietins (CEDOSE) trial testing the effects of fixed dose ESA combinations, should provide additional evidence whether the ESA dose itself or the targeted hemoglobin level mediates the increased risk of adverse renal and CV outcomes.(44) Nevertheless, the lack of renal and CV protective effect despite increasing hemoglobin levels in the TREAT trial indicate that hemoglobin is a poor risk/biomarker to follow the response of ESA therapy.

C-reactive protein

In recent years, it has been postulated that chronic low-grade tissue inflammation may play a critical role in initiation and progression of atherosclerosis and diabetic renal and CV injury. Several studies have demonstrated that measurement of low-grade inflammatory risk/biomarkers, among them high sensitivity C-reactive protein (hs-CRP), improves CV risk stratification, particularly in those already at intermediate or high CV risk.(45-48).The predictive ability of hs-CRP goes beyond CV risk prediction. Laaksonen and Brantsma have provided evidence that the presence of elevated concentrations of hs-CRP in apparently healthy individuals is associated with increased risk for *de*

novo type 2 diabetes.(49, 50)

Statin therapy has greatest effects in the presence of inflammation. Several studies show that statin therapy reduces hs-CRP.(51, 52) In trials of patients with coronary disease and acute coronary syndrome, the benefits of statin therapy relate at least in part to their effect on hs-CRP reduction.(53, 54) The recent Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trial (JUPITER) trial was designed to study the effect of rosuvastatin in people with normal Low Density Lipoprotein-levels (LDL) but increased hs-CRP concentrations. The trial showed that rosuvastatin lowered LDL-cholesterol and hs-CRP in the short-term and markedly lowered the long-term risk for CV events.(55) In this trial rosuvastatin both lowered LDL-cholesterol and hs-CRP. Thus whether the reductions in hs-CRP or LDL or combined (or another undiscovered risk/biomarker) were the driving parameter for CV protection could not be established. A post-hoc analysis recently provided further insight into this topic. This analysis demonstrated that in patients assigned to rosuvastatin who achieved LDL-cholesterol of less than 1.8 mmol/L and hs-CRP of less than 2 mg/L at 1 year had substantially lower risk for CV events compared to those who achieved neither target or only LDL-cholesterol less than 1.8 mmol/L (Table 1 and Figure 1).(56) The correlation between the achieved LDL-cholesterol and hs-CRP concentrations was small indicating that only a small part of the achieved hs-CRP concentration could be explained by the achieved LDL-cholesterol. These data suggest that the extent to which statins lower hs-CRP and LDL-cholesterol in the short term determines the degree of long-term CV protection.

As was the case for the post-hoc analyses of albuminuria trials described above, the JUPITER trial did not provide direct evidence that monitoring of hs-CRP to guide the intensity of statin therapy resulted in improved CV outcomes. An alternative JUPITER design in which one group of patients is assigned to intensive hs-CRP targeting while the control group receives usual care would have provided direct evidence of the value of a hs-CRP targeted intervention approach. Until such data become available, the efficacy of improving health outcomes using hs-CRP as a target is not proven.

NT-ProBNP

Amino-terminal pro-B-type natriuretic peptide (NT-proBNP) is a risk/biomarker of the cardiac response to volume overload. In the setting of increased volume expansion, pre-proBNP is released and subsequently converted into active BNP and inactive NT-proBNP. Studies in patients with diabetes and diabetic nephropathy have shown that NT-proBNP is an important prognostic marker

for CV events and all cause mortality.

ACEi and ARBs reduce plasma concentrations of NT-proBNP in post-myocardial infarction and congestive heart failure patients. Anand et.al demonstrated that the ARB valsartan caused a sustained reduction in brain natriuretic peptide (BNP) within 4 months of therapy. Individuals in whom BNP concentrations were attenuated during the first 4 months had markedly lower risk for long-term CV events than those in whom BNP rose during the first months of therapy (Figure 1).(57) The Steno-2 trial in patients with type 2 diabetes showed that intensive targeting of multiple CV risk factors reduced NT-proBNP during follow-up compared to conventional therapy.(58) Interestingly, the magnitude of NT-proBNP reduction during the first 2 years of treatment was associated with the reduction in CV risk during the subsequent 6 years of follow-up (Table 1).

These results suggest that attenuating NT-proBNP levels in the short-term improves long-term health outcomes. However, as mentioned above, such post-hoc analyses do not provide direct evidence that a NT-proBNP targeted approach confers long-term renal and/or CV protection. A couple of RCTs have been conducted to test the hypothesis that a NT-proBNP targeted approach improves long-term CV outcomes. The earlier trials showed promising effects in terms of CV outcomes. However these trials were performed in small populations and had limited duration of follow-up.(59, 60) Pfisterer et. al. recently investigated a NT-proBNP guided strategy in a larger cohort of 499 heart failure patients of whom approximately one third had diabetes.(61) The trial failed to achieve significant differences in NT-proBNP concentrations between the two treatment groups, although NT-proBNP concentrations were numerically lower in the targeted group. After 18 months, no CV benefit was observed in patients assigned to NT-proBNP targeted therapy compared to those receiving conventional symptom-guided therapy. Thus, the value of specific targeting of NT-proBNP on top of symptom guided therapy seems limited, at least in this population of patients with heart failure, despite the unquestionable diagnostic and prognostic significance of NT-proBNP. Whether a NT-proBNP targeted approach confers renal or CV protection in other populations warrants further research.

Targeting multiple risk/biomarkers

Since the etiology of diabetes is multi-factorial, it has been suggested that a multi-factorial approach targeting the various pathways involved in the pathogenesis of diabetes simultaneously would lead to more salutary long-term outcomes. One of the few studies that evaluated systematically the long-term effects of a multi-factorial treatment strategy in type 2 diabetes is the Steno-2 trial. (3) The intensive multi-factorial intervention consisted of pharmacological agents targeting blood

pressure, HbA1c, lipid concentrations and of behavioral modifications including smoking cessation and diet changes. After 4 years follow-up, multi-factorial intervention slowed the progression of nephropathy and retinopathy. In addition, the long-term follow-up data of this study demonstrated that patients assigned to intensive multi-factorial intervention had a considerable significantly lower risk for CV events and mortality, highlighting the importance of a multi-factorial treatment regimen (table 1).(62)

A new class of oral glucose lowering agents is currently under development targeting multiple risk/biomarkers. These drugs are designed as selective inhibitors of the sodium-glucose co-transporter (SGLT2). The SGLT2 receptor mediates glucose (and sodium) reabsorption in the proximal tubule of the kidney.(63) Randomized controlled trials have shown that blockade of the SGLT2 receptor increases urinary glucose excretion and lowers HbA1c.(64) Intriguingly, these drugs appear to have favorable effects on other renal and CV risk/biomarker as well, including blood pressure and body weight reduction.(64) Thus, multiple renal and CV risk/biomarkers are targeted with a single drug. Supposedly, these multiple favorable effects result in substantial renal and CV protection. A couple of trials are currently underway testing the efficacy and safety of SGLT2 inhibitors on renal and CV endpoints. The results of these trials are eagerly awaited.

Other novel risk/biomarkers

Besides the risk/biomarkers discussed above, the effects of different pharmacological agents on other single risk/biomarkers predicting CV and renal disease have been tested. Several studies have shown that RAAS inhibiting therapy can modify risk/biomarkers reflecting endothelial and tubular damage and inflammation.(65-71) Furthermore, metformin has been reported to lower inflammation biomarkers.(72) However, to the best of our knowledge, all these studies were performed in small populations with only limited duration of follow-up. Therefore, the impact of short-term treatment induced changes in these risk/biomarkers on long-term hard renal and CV outcomes cannot be ascertained. Further studies are clearly warranted to test whether therapeutic interventions aimed at targeting these novel risk/biomarkers afford long-term protection. If so, the clinical utility of these novel risk/biomarkers in the management of diabetic renal and CV complications will increase.

Conclusion

Over the last two decades, a vast number of risk/biomarkers predicting renal and CV complications have been discovered. However, few studies have systematically tested whether short-term treatment-induced changes in these risk/biomarkers relate to long-term protection. Moreover,

whether a targeted approach, directed at changing a panel of multiple novel risk/biomarkers, will improve long-term renal and CV outcome remains an unsolved question. The examples with targeting single novel risk/biomarkers, such as hemoglobin and NT-proBNP, teach us that although a risk/biomarker may have superior prognostic ability, such ability does not automatically imply that specific targeting of and changing such a risk/biomarker will improve long-term outcome. Thus, to establish the clinical utility of novel risk/biomarkers in guiding treatment intensity, specific protocols need to be developed and employed to demonstrate that targeting single (or multiple) novel risk/biomarkers improves long-term health outcomes.

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CHAPTER 3

Effect of a reduction in serum uric acid during losartan treatment on renal outcomes in patients with type 2 diabetes and nephropathy: a post-hoc analysis of the RENAAL trial

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Hypertension. 2011;58:2-7.

Abstract

Background: Emerging data show that increased serum uric acid (SUA) concentration is an independent risk factor for End-Stage-Renal-Disease (ESRD). Treatment with the antihypertensive drug losartan lowers SUA. Whether reductions in SUA during losartan therapy are associated with renoprotection is unclear. We therefore tested this hypothesis.

Methods: In a post-hoc analysis of 1342 patients with type 2 diabetes and nephropathy participating in the RENAAL trial, we determined the relationship between month 6 change in SUA and renal endpoints, defined as a doubling of serum creatinine or ESRD.

Results: Baseline SUA was 6.7 mg/dl in placebo and losartan treated subjects. During the first 6 months, losartan lowered SUA by -0.16 mg/dl (95%CI -0.30 to -0.01; p=0.031) as compared with placebo. The risk of renal events was decreased by 6% (95%CI 10% to 3%) per 0.5 mg/dl decrement in SUA during the first 6 months. This effect was independent of other risk markers, including eGFR and albuminuria. Adjustment of the overall treatment effects for SUA attenuated losartan's renoprotective effect from 22% (95%CI 6 to 35) to 17% (95%CI 1 to 31), suggesting that about one-fifth of losartan's renoprotective effect could be attributed to its effect on SUA.

Conclusion: Losartan lowers SUA levels compared to placebo treatment in patients with type 2 diabetes and nephropathy. The degree of reduction in SUA is subsequently associated with the degree in long-term renal risk reduction and explains part of losartan's renoprotective effect. These findings support the view that SUA may be a modifiable risk factor for renal disease.

Introduction

Over the past decades, serum uric acid has emerged as a cardiovascular risk marker. Increased serum uric acid has been shown to predict the risk of hypertension, diabetes, and cardiovascular disease.(1-3) More recent data also point to serum uric acid as a risk marker for progression of chronic kidney disease.(4, 5)

These observations raise the question as to whether interventions that lower uric acid could confer cardiovascular or renal protection. In this respect, the angiotensin receptor blocker losartan is of potential interest. The drug has been clearly demonstrated to be renoprotective in patients with diabetic nephropathy, this effect largely attributed to its effects on blood pressure and/or proteinuria/albuminuria.(6) However, it is unclear whether other off-target effects of the drug could contribute to the ultimate improvement in renal outcome with this agent. Importantly, previous studies have shown that losartan lowers serum uric acid. This hypouricemic effect does not occur with other ARBs,(7) and appears to be largely mediated through reductions in the level of human urate transporter 1 (URAT1) and decreased net urate reabsorption in the proximal tubule.(8-10)

With respect to cardiovascular endpoints, a sub-analysis from The Losartan Intervention For Endpoint reduction in hypertension (LIFE) trial showed that the superior effect of losartan could be partly explained by its effect on serum uric acid.(11) Whether the same holds true for the long term renoprotective effect of losartan is unknown but is worth investigating in the context of the increased body of evidence linking uric acid to the progression of chronic kidney disease.(12) The aim of the present study, therefore, was to assess whether losartan induced changes in uric acid during initial months of therapy are associated with decreased (long-term) risk of readily measurable renal outcomes in patients with type 2 diabetes and nephropathy.

Methods

Study Design

The Reduction of Endpoints in non-insulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) trial was a multinational, randomized, double-blind trial that compared the effects of losartan versus placebo in addition to conventional antihypertensive medication in patients with type 2 diabetes and nephropathy. Patients had serum creatinine levels between 1.3 and 3.0 mg/dl (1.5 to 3.0 mg/dl for males more than 60 kg). The study was performed in 250 centers in 28 countries and involved 1513 patients. The study design, the inclusion/exclusion criteria, and the treatment protocol have been reported previously.(13) In short, after a 6-week screening phase, patients were randomized to either losartan 100 mg or placebo. Additional

antihypertensive medications (calcium channel blockers, β-blockers, centrally acting agents, and diuretics, excluding angiotensin-converting enzyme inhibitors or other angiotensin receptor antagonists) were permitted in order to achieve the blood pressure goal of < 140/90 mmHg (systolic/diastolic). All patients signed informed consent before enrollment, and the local Institutional Review Board of each participating center approved the study. The mean duration of follow-up was 3.4 years. The RENAAL trial is registered with Clinical trials.gov, identifier: NCT 00308347.

Measures and outcomes

In this study, we performed a post-hoc analysis of all subjects with uric acid measurements included in the RENAAL trial. Blood pressure, serum uric acid, serum creatinine and albuminuria were measured at a 3 monthly basis and HbA1c was measured every six months for the duration of the study. Albuminuria was assessed as the ratio of albumin to creatinine concentrations from a first-morning-void urine sample. The MDRD formula was used to estimate GFR (eGFR).(14) We assessed the relationship between change in serum uric acid level at month 6 and renal outcomes. The change from baseline to month 6 was chosen since this is the earliest time point at which most variables of interest were available, the treatment effects were considered to be fully manifest, and relatively few renal events occurred before month 6.(15) Changes in serum uric acid, blood pressure, albuminuria and HbA1c were calculated as baseline minus month 6.

The primary renal outcome was defined as a composite of a confirmed doubling of serum creatinine (DSCR) or End Stage Renal Disease (ESRD). The latter was defined as the need for chronic dialysis or renal transplantation. All endpoints were adjudicated by a blinded endpoint committee using rigorous guideline definitions.

Statistical Analysis

Patients with serum uric acid measurements at baseline and month 6 were included in the present analysis. Mean serum uric acid at each visit during follow-up was calculated in both the losartan and placebo group. Patient characteristics were summarized according to tertiles of month 6 changes in serum uric acid. To identify parameters associated with a change in serum uric acid at month 6, a multivariate logistic regression model was used. Baseline characteristics as well as month 6 changes in systolic and diastolic blood pressure, HbA1c, log transformed albuminuria, and eGFR were included in the multivariate model. A backward selection procedure was used for selection of covariates for the final model (α=0.1).

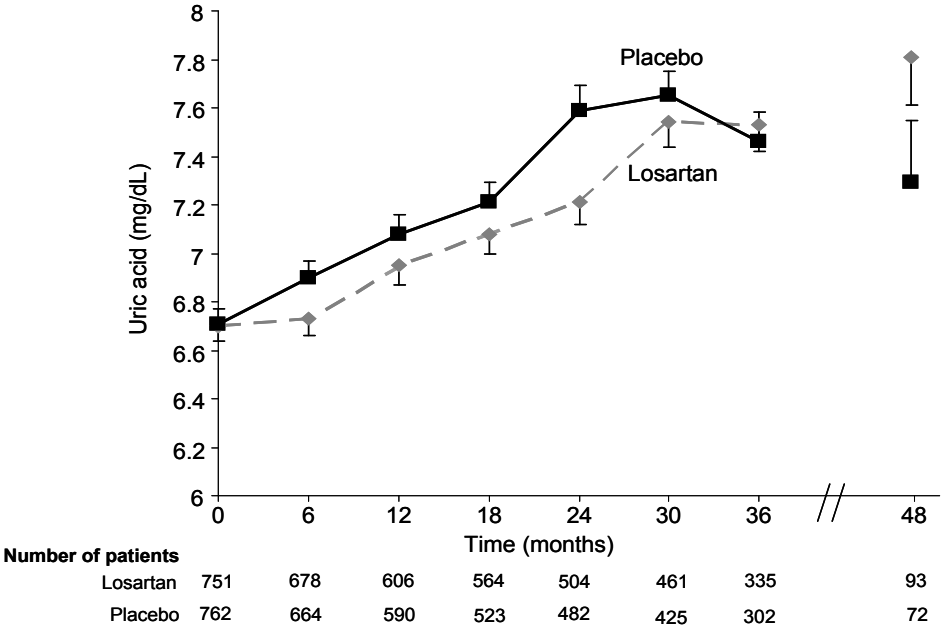
The proportion of patients without renal events was estimated using the Kaplan-Meier

procedure. Multivariate Cox regression analyses were performed to determine whether changes in serum uric acid were independently associated with renal outcomes. Changes in serum uric acid were included in the Cox model as a continuous variable. All analyses were adjusted for risk markers that showed a statistically significant association with month 6 change in uric acid. These included: age, gender, treatment assignment (losartan or placebo), eGFR, systolic blood pressure, log transformed albuminuria, serum albumin, ACEi or ARB use at baseline, and changes in log transformed albuminuria and eGFR. Finally, the contribution of therapy induced changes in serum uric acid on losartan's renoprotective effect was assessed by time-varying Cox regression models. Relative risk reductions are described in the text as percentage reductions ([1–hazard ratio] ×100). Analyses were conducted with SAS (version 9.2; SAS Institute, Cary, NC). A p-value < 0.05 was considered to indicate statistical significance.

Results

A total of 1342 subjects were involved in the present analysis. In the losartan group, the mean serum uric acid remained 6.7 mg/dl during the first 6 months of therapy. By contrast, in the placebo group, the mean serum uric acid increased from 6.7 mg/dl at baseline to 6.9 mg/dl at month 6, resulting in a mean group difference of -0.16 mg/dl (95% CI -0.30 to -0.01; p=0.031) (figure 1). The level of serum uric acid in the placebo group continued to increase from month 6 onwards. Likewise, the serum uric acid level also started to rise at month 6 in the losartan group. The "apparent" fall observed at 36 month in the placebo group is likely to be linked to "drop-out" of patients in the placebo group with high serum uric acid levels. Patients were subsequently classified into tertiles according to the change in serum uric acid at month 6 (table 1). Relevant baseline characteristics were not different among the tertile groups apart from serum uric acid and albuminuria which were higher and respectively lower in patients who had a decrease in serum uric acid. In addition, these patients had a smaller reduction in systolic blood pressure and albuminuria at month 6.

Figure 1: Mean uric acid level during follow-up among patients in the losartan and placebo group
Bars represent standard errors



Number of patients								
Losartan	751	678	606	564	504	461	335	93
Placebo	762	664	590	523	482	425	302	72

Table 1: Characteristics of the overall RENAAL population by treatment allocation and by month 6 change in uric acid

Characteristics	Placebo (n=664)	Losartan (n=678)	Tertiles of change in uric acid		
			Uric acid decrease ≥0.5 mg/dl (n=457)	-0.5< ΔUric acid <0.5 mg/dl (n=435)	Uric acid increase ≥ 0.5 mg/dl (n=450)
Age, yrs	60.2 (7.6)	60.0 (7.3)	60.3 (7.2)	60.2 (7.6)	59.9 (7.5)
Male, n (%)	421 (63.4)	422 (62.2)	296 (64.5)	270 (62.1)	277 (61.6)
Race, n (%)					
White	327 (49.3)	322 (47.5)	228 (49.9)	212 (48.7)	209 (46.4)
Black	92 (13.9)	109 (16.1)	62 (13.6)	65 (14.9)	74 (16.4)
Hispanic	120 (18.1)	124 (18.3)	90 (19.7)	80 (18.4)	74 (16.4)
Asian	117 (17.6)	114 (16.8)	70 (15.3)	72 (16.6)	89 (19.8)
Other	8 (1.2)	9 (1.3)	7 (1.5)	6 (1.4)	4 (0.9)
Systolic BP, mmHg	152.9 (20)	152.0 (19)	151.1 (19.1)	152.1 (19.8)	154.2 (19.2)
Diastolic BP, mmHg	82.3 (10)	82.4 (10)	82.3 (10.5)	82.1 (10.3)	82.7 (10.4)
Total cholesterol, mg/dl	227.9 (56)	225.7 (55)	224.6 (55.1)	226.3 (52.4)	229.5 (58.0)
HbA1C, %	8.4 (1.6)	8.5 (1.6)	8.3 (1.6)	8.5 (1.7)	8.5 (1.6)
Serum uric acid, mg/dl	6.7 (1.7)	6.7 (1.7)	7.4 (1.8)	6.4 (1.5)	6.4 (1.5)*
Hemoglobin, mg/dl	12.4 (1.8)	12.5 (1.8)	12.6 (1.9)	12.5 (1.8)	12.4 (1.8)
Urinary ACR, mg/g	1261 (568-2475)	1168 (538-2540)	947 (475-1964)	1246 (593-2682)	1369 (693-2831)*
eGFR, ml/min/1.73m^2	39.8 (12.7)	39.5 (11.8)	39.8 (12.2)	39.9 (12.4)	39.2 (12.2)
Serum creatinine, mg/dl	1.9 (0.5)	1.9 (0.5)	1.9 (0.5)	1.9 (0.5)	1.9 (0.5)
Serum albumin, mg/dl	3.8 (0.4)	3.8 (0.4)	3.7 (0.4)	3.8 (0.4)	3.7 (0.4)
Other treatments, n (%)					
ACEi or ARB	329(49.6)	368 (54.3)	237 (51.9)	235 (54.0)	225 (50.0)
β-blockers	122 (18.4)	128 (18.9)	102 (22.3)	68 (15.6)	80 (17.8)
Calcium channel blockers	484 (72.9)	488 (72.0)	323 (70.7)	308 (70.8)	341 (75.8)
Diuretics	384 (57.8)	394 (58.1)	282 (61.7)	236 (54.3)	260 (57.8)
Follow-up characteristics					
Change in uric acid, mg/dl	0.2 (1.4)	0.0 (1.3)	-1.3 (0.8)	0.0 (0.2)	+1.5 (0.9)
Change in systolic BP, mmHg	-0.3 (20)	-5.4 (19)	-0.4 (19)*	-2.3 (19)	-6.0 (21)
Change in diastolic BP, mmHg	-0.7 (11)	-2.7 (10)	-0.6 (10)	-1.2 (9)	-3.5 (10)
Change in UACR, %	+4.7	-28.8	+2.6	-13.2	-29.7*

*P<0.05, tests for trend among tertiles of month 6 serum uric acid change

Mean (SD) or numbers of patients (%) were provided for normal distributed continuous variables and categorical variables respectively. Due to the skewed distribution of the albumin:creatinine ratio, urinary albumin:creatinine ratio is presented as median (interquartile range).

To convert the values of serum uric acid to micromoles per liter, multiply by 59.48. To convert the values of serum creatinine to micromoles per liter, multiply by 88.4. To convert the values for cholesterol to millimoles per liter, multiply by 0.0259.

To investigate the parameters associated with a change in serum uric acid at month 6, a multivariate linear regression was performed in the overall population. Allocation to losartan therapy was independently associated with a larger fall in uric acid at month 6. Furthermore, higher baseline serum uric acid, eGFR, and serum albumin as well as a larger reduction in eGFR and a smaller reduction in albuminuria were significantly associated with a larger decrease in serum uric acid at month 6 (table 2).

Table 2: Covariates associated with a change in serum uric acid at month 6

Risk markers	Beta	X^2	P value
Baseline uric acid	0.253	191.6	<0.001
Change urinary albumin:creatinine ratio	0.526	148.4	<0.001
Treatment assignment (losartan or placebo)	0.477	57.6	<0.001
Change eGFR	-0.032	41.5	<0.001
Baseline eGFR	0.010	13.1	0.002
Baseline serum albumin	0.263	12.6	0.003
Baseline ACEi or ARB use	-0.143	5.5	0.049
Baseline systolic blood pressure	-0.004	4.8	0.067
Gender	0.134	4.1	0.090

The natural log transformed value of urinary ACR and change in natural log transformed urinary ACR was used in all regression analyses. Covariates which showed a p-value less than 0.1 in the multivariate analysis are presented in the table. Covariates are ordered by decreasing significance based on the X^2 statistics.

Figure 2 shows the proportion of patients free of renal events during follow-up according to the change in serum uric acid. A total of 463 renal events occurred during follow-up. Those subjects with a decrease in serum uric acid more than 0.5 mg/dl at month 6 had the lowest risk of developing renal endpoints (figure 2). Subsequently, hazard ratios were calculated for finer categories of change in uric acid. After controlling for baseline and change in other risk factors we observed an almost linear relationship between the change in uric acid and renal outcome (figure 3), so that each 0.5 mg/dl reduction in serum uric acid during the first 6 months was associated with a reduction in the risk of DSCR / ESRD of 6% (95%CI 10 to 3; p<0.001).

Figure 2: Kaplan–Meier curves for renal outcomes (DSCR or ESRD). The renal event rates in subjects with a month 6 reduction in SUA ≥0.5 mg/dL, serum uric acid (SUA) change between and 0.5 mg/dL, or an SUA increase ≥0.5 mg/dL were, respectively, 9.5, 12.3, and 14.3 events per 100 patient-years

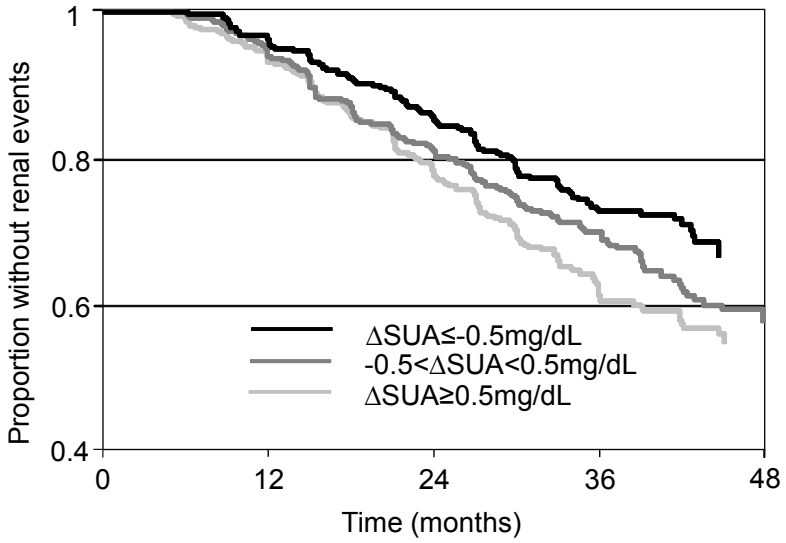
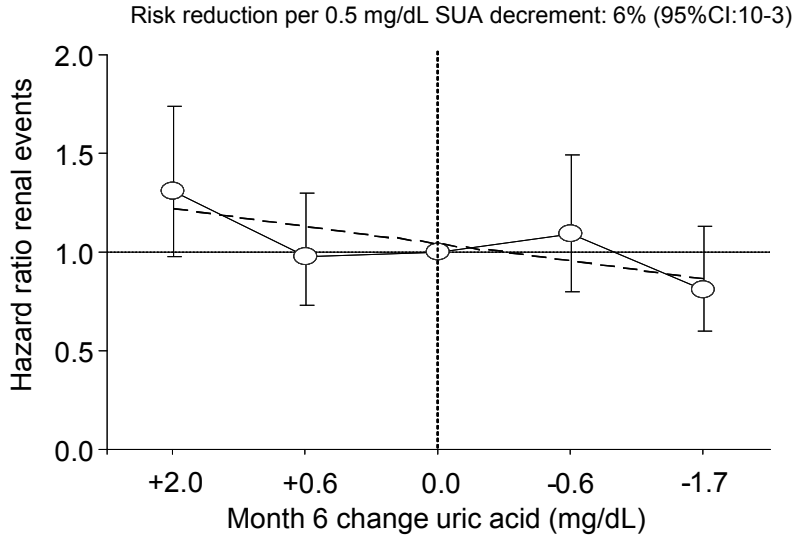


Figure 3: Hazard ratios for incident renal outcomes (DSCR or ESRD) as function of month 6 change in serum uric acid. The relation is corrected for baseline and change in other risk marker



To investigate how much of losartan's renoprotective effect could be attributed to its effect on serum uric acid, we analyzed the impact of a reduction in serum uric acid over time on losartan's renoprotective effect. When the treatment effect was adjusted for the residual serum uric acid (the last measurement prior to the occurrence of the renal endpoint) the treatment effect of losartan on the DSCR / ESRD endpoint attenuated from 22% (95%CI 6 to 35) to 17% (95%CI 1 to 30); that is about 4% out of 22% (one fifth) of the benefit of losartan could be attributed to its effect on serum uric acid.

Discussion

This study demonstrates that losartan treatment in patients with type 2 diabetes and nephropathy lower serum uric acid levels compared with placebo. Whereas serum uric acid increased in the placebo group, this effect was attenuated with losartan in the treated group. A significant lower risk for renal events was observed per decrement in serum uric acid during the first 6 months, and the association remained statistically significant (and unchanged) after adjustment for a broad range of known risk factors. The effect of losartan on serum uric acid explained approximately 20% of its renoprotective effect.

This is the first study that directly shows that the effect of losartan on serum uric acid is associated with renal risk reduction. Thus, the effect on uric acid by losartan appears not only relevant for cardiovascular outcomes as reported in the LIFE study,(11) but also for the renal outcome. The estimated contribution of serum uric acid to losartan cardiovascular protective treatment effects was calculated to be 29% in the LIFE trial. In the present population, the contribution of serum uric acid to losartan's renoprotective effect was estimated to be 22%.

The mechanisms through which losartan exerts its hypouricemic effect are well described. The proximal tubule has been identified as the primary location of uric acid secretion and reabsorption. A central role in proximal tubule urate reabsorption has been ascribed to URAT1. URAT1 is located in the lumen of proximal tubule cells and reabsorbs uric acid (as urate) in exchange for intracellular anorganic anions. Losartan increases urate excretion by inhibition URAT1 mediated renal tubule urate reabsorption. Early studies in the healthy population demonstrated that the peak uricosuric effect was already observed two to four hours after administration.(16, 17) The time course of this effect suggests that it is losartan itself rather than its active metabolite that blocks URAT1 and causes the reduction in serum uric acid. Theoretically, the distinct uricosuric effect of losartan could lead to increases in urinary uric acid concentration which could lead to supersaturation of uric acid and in the extreme case precipitate uric acid nephropathy. However, the

risk of development of uric acid crystals during losartan therapy is reduced due to the drug's urinary alkalinizing effects. Treatment with losartan raises urinary pH which is attributed to the blockade of angiotensin II induced stimulation of bicarbonate reabsorption. This increase in urinary pH offsets the formation of uric acid crystals and reduces the risk of acute uric acid nephropathy.(18)

Emerging evidence demonstrate an association between serum uric acid and adverse renal outcomes.(12) Whether this relationship is causal is unclear. Indeed, whether serum uric acid is a marker of renal function decline or a risk factor for progressive renal function loss remains a matter of ongoing debate. In the kidney serum uric acid is filtered, secreted and reabsorbed. As glomerular filtration rate (GFR) declines, the fractional excretion of uric acid increases. However, this process does not completely counterbalance the fall in GFR. Consequently, serum uric acid levels start to rise. It is therefore reasonable to suggest that changes in serum uric acid are a result of renal disease and have no direct pathogenic role. However, a series of experimental and epidemiological studies have challenged this view. Recent experimental studies have shown that increased uric acid levels increase activity of the renin-angiotensin-aldosterone-system,(19) stimulate renal inflammation,(20) enhance endothelial dysfunction,(21) and impair renal autoregulation resulting in glomerular (and systemic) hypertension.(22) Each of these effects contributes to the initiation and progression of renal disease. In addition, epidemiological studies consistently show that increased serum uric acid levels predict renal function decline, independently of other renal or cardiovascular risk factors. For example, Hovind et.al. recently showed that increased uric acid is independently associated with development of nephropathy, albeit that study was performed in a cohort with type 1 diabetes. (1) These experimental and clinical studies support the view that uric acid may be involved in the pathogenesis of renal disease.

The most compelling way to evaluate whether uric acid is a marker or risk factor for renal disease is to evaluate whether "direct" therapy that lowers uric acid confers renoprotection. A couple of studies have highlighted the relevance for renal outcomes of manipulating serum uric acid concentrations. It appears that reductions in serum uric acid conferred by allopurinol slow down progressive renal function loss in diabetic and non-diabetic patients with chronic kidney disease. (23, 24) In addition, treatment of asymptomatic hyperuricemia has been reported to improve renal function even in subjects with normal renal function.(25) The results of our analysis showing that one-fifth of losartan's renoprotective effect could be attributed to serum uric acid provide further support for the postulate that treatment induced reductions in serum uric acid are associated with renoprotection, independent of baseline or changes in renal function. Furthermore, our study indicates that it is reduction of uric acid *per se* that is important rather than the specific treatment

strategy employed.

Serum uric acid increased in the placebo group during the trial and started to increase after 6 months in the losartan group. A similar trend of changes in serum uric acid over time has been observed in the LIFE trial.(11) In the RENAAL trial, GFR fell in the placebo and losartan groups, a decline of 5.2 and 4.4 ml/min/1.73m^2/year, respectively. We cannot exclude that the longitudinal increase in serum uric acid reflects, at least in part, a reduction in GFR over time. An alternative possibility is the possible interference of other drugs influencing serum uric acid. The proportion of patients receiving a diuretic increased from 58% at baseline to 71% at month 6 and to 84% at the time of the primary renal endpoint. The increasing use of concomitant diuretic therapy, which increases serum uric acid, could be an alternative explanation for the increase in serum uric acid observed during the trial. Since the proportion of patients receiving diuretics was similar between the placebo and losartan group at baseline, month 6, and at the end of the trial, it is unlikely that concomitant diuretic use has confounded our findings. A final explanation may relate to the effects of losartan on uric acid handling during prolonged therapy. In a previous study, the acute effects of losartan on uric acid excretion after 3 weeks losartan therapy were less pronounced compared with the effects after the first dose was administered.(18) This suggests that the effects of losartan on uric acid excretion wane off over time once a new steady state has been achieved.

The data from our study suggest that losartan, registered as a blood pressure lowering drug, confers additional renal protection partly through its effect on serum uric acid. Other drugs used in renal and cardiovascular risk management appear to lower serum uric acid as well. Fenofibrate, registered as a lipid lowering drug, has been shown to decrease serum uric acid. These effects were independent of changes in lipid parameters indicating that the drug itself exerts uricosuria.(26) Furthermore, fenofibrate has been reported to have certain renal benefits including on albuminuria.(27) Another lipid lowering drug, atorvastatin, has been shown to have hypouricemic effects as well, irrespective of the drug's effect on lipid parameters.(28) Whether the effects of these drugs on uric acid excretion contribute to their long-term renal and/or cardiovascular protective effects are uncertain. On the contrary, drugs that increase serum uric acid may adversely influence renal and cardiovascular risk. It is known that diuretics, also registered as blood pressure lowering drugs, increase serum uric acid. In this respect, a post-hoc analysis of the SHEP trial demonstrated that the cardiovascular protective effect of diuretic therapy was restricted to those individuals in whom serum uric acid increased less than 1 mg/dl after one year therapy.(29) Thus, when estimating the effects of a drug on renal or cardiovascular endpoints using risk markers, the effect of the drug on all risk markers including serum uric acid should be taken into account rather than focusing on the

marker the drug is registered for.

Limitations of this study include the post-hoc nature of the analyses. Although our data are derived from a double blind, placebo controlled randomized trial, the analyses according to change in serum uric acid are no longer randomized. Although we adjusted for all available baseline covariates and changes in covariates, residual confounding cannot be completely excluded. Unfortunately, 24-hour urate excretion was not measured in RENAAL participants. This precludes the possibility to determine the fractional excretion of uric acid during losartan therapy. The reduction in uric acid in subjects with the highest baseline uric acid could indicate a regression to the mean phenomenon. However, the fact that we adjusted our multivariate analyses for baseline uric acid and the fact that the month 6 uric acid (residual uric acid) remained an independent predictor for the primary renal outcome makes this assumption as an explanation for our findings less likely. Finally, the results of this study can only be generalized to the, admittedly large, population of patients with type 2 diabetes and nephropathy.

In conclusion, losartan lowers serum uric acid levels, when compared to placebo treatment in patients with type 2 diabetes and nephropathy. This change in serum uric acid is independently associated with a lower risk of doubling of serum creatinine or end stage renal disease such that approximately one fifth of losartan's renoprotective effect could be attributed to serum uric acid. These data indicate that a reduction in serum uric acid observed during the initial months after starting losartan contributes to its renoprotective effect.

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CHAPTER 4a

Increased serum potassium affects renal outcomes: a post-hoc analysis of the RENAAL trial

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Abstract

Aims: To assess the effect of an ARB on serum potassium and the effect of a serum potassium change on renal outcomes in patients with type 2 diabetes and nephropathy.

Methods: We performed a post-hoc analysis in patients with type 2 diabetes participating in the RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan Study) trial. Renal outcomes were defined as a composite of doubling serum creatinine or end stage renal disease.

Results: At month 6, 259 (38.4%) and 73 (10.8%) patients in losartan and 151 (22.8%) and 34 (5.1%) patients in placebo group presented serum potassium ≥5.0 mmol/L and ≥5.5 mmol/L, ($p < 0.001$) respectively. Losartan was an independent predictor for serum potassium ≥5.0 mmol/L at month 6 (OR 2.8; 95%CI 2.0-3.9). A serum potassium at month 6 ≥5.0 mmol/L was in turn associated with increased risk for renal events (HR 1.22; 95%CI 1.00-1.50), independently of other risk factors. Adjustment of the overall treatment effects for serum potassium augmented losartan's renoprotective effect from 21% (6%–34%) to 35% (20%–48%), suggesting that the renoprotective effects of losartan are offset by its effect on serum potassium.

Conclusions: In this study we found that treatment with the ARB losartan is associated with a high risk of increased serum potassium levels which is in turn associated with an increased risk of renal outcomes in patients with diabetes and nephropathy. Whether additional management of high serum potassium would further increase the renal protective properties of losartan is an important clinical question.

Introduction

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease (ESRD) in western societies. As the prevalence of diabetes is increasing, ESRD accounts for substantial morbidity and mortality.(1, 2)

Angiotensin receptor blockers (ARBs) have several important beneficial effects in patients with diabetes and nephropathy, such as decreasing systemic blood pressure and reducing albuminuria. These effects are related to long-term renal protection.(3, 4) However, these beneficial effects are accompanied by a so-called side effect of ARBs, induction of a rise in serum potassium levels even leading to hyperkalemia. This may in turn lead to detrimental long-term effects.(5) The risk of hyperkalemia is particularly high in patients with diabetes as these patients already have reduced aldosterone production secondary to renin deficiency, the so-called hyporeninemic hypoaldosteronism syndrome.(6, 7) Diabetes, low renal function and use of renin-angiotensin-aldosterone system (RAAS) inhibitors are independent factors that increase serum potassium level. In combination, these factors pose patients at even higher risk of hyperkalemia.(8)

Increased serum potassium levels are associated with increased risk for cardiovascular (CV) morbidity and mortality. Hyperkalemia as a result of ARB therapy has been related to worse CV outcomes.(9) However, the relationship between change in serum potassium levels in response to RAAS therapy and renal outcomes is not well established. Therefore, we assessed the relationship between ARB treatment, serum potassium levels and renal outcomes in patients with type 2 diabetes and nephropathy participating in the RENAAL trial (Clinical trials.gov identifier: NCT 00308347).

Methods

Study Design

The RENAAL trial was a multinational, randomized, double-blind trial that compared the effects of losartan versus placebo in addition to conventional antihypertensive medication in patients with type 2 diabetes and nephropathy. Patients had serum creatinine levels between 1.3 and 3.0 mg/dL (1.5 to 3.0 mg/dL for males more than 60 kg). The study was performed in 250 centers in 28 countries and involved 1513 patients. The study design, the inclusion/exclusion criteria, and the treatment protocol have been reported previously.(3, 10) In short, after a 6-week screening phase, patients were randomized to either losartan 50 mg (titrated to 100 mg after 4 weeks) or placebo. Additional antihypertensive medications (calcium channel blockers, β-blockers, centrally acting agents, and diuretics, excluding angiotensin-converting enzyme inhibitors or other angiotensin receptor antagonists) were permitted to reach the blood pressure goal of < 140/90 mmHg (systolic/diastolic).

All patients signed informed consent before enrollment, and the local Institutional Review Board of each participating center approved the study. The mean duration of follow-up was 3.4 years. Blood pressure, serum potassium level, serum creatinine and albumin:creatinine ratio were measured at 1st month, 3rd month, and then every three months until the end of the study.

Change in serum potassium and outcomes

In this study, we performed a post-hoc analysis of all subjects with potassium measurements included in the RENAAL trial. We assessed the relationship between serum potassium level and renal outcomes in two ways. First, we assessed the relationship between serum potassium and renal outcomes at month 6. The month 6 values were chosen since the treatment effects were considered to be fully present and relatively few renal events occurred before month 6. The month 6 serum potassium level was classified into two categories: <5.0 mmol/L (reference) and ≥5.0 mmol/L.(11) We selected this threshold (instead of the clinical accepted value of 5.5 mmol/L) because the risk of adverse renal outcomes started to increase from 5.0 mmol/L, and a small number of patients reached serum potassium levels ≥5.5 mmol/L in our population. Since a single elevated potassium measurement may be an erroneous finding, we also assessed the relationship between persistent drug induced serum potassium at month 6 and 9 and its association with renal outcome. These subjects were either compared with those with a single elevated serum potassium measurement at month 6 or 9, or compared with those without increases in serum potassium above 5.0 mmol/L during the first 9 month of follow-up.

In the second approach, we calculated the average serum potassium concentration during follow-up and explored the relationship between the average serum potassium level during follow-up with renal outcomes. The average serum potassium concentration, as well as average levels of other relevant covariates was calculated as the mean of the first month and each consecutive third month potassium value until the occurrence of the renal event. This approach was chosen since it more accurately reflects the risk of a subject to exposure to a high serum potassium load for a definite period of time than a single elevated measure. Renal outcomes were defined as a composite of doubling serum creatinine (DSCR) or end stage renal disease (ESRD), and as DSCR and ESRD individually. All endpoints were adjudicated by a blinded end point committee using rigorous guideline definitions.

Statistical Analysis

Differences among patient subgroups were evaluated by using Chi-square test or t-test, as appropriate. Mean serum potassium level at each visit during follow-up, as well as the proportion of patients with month 6 potassium level ≥5.0 mmol/L and ≥5.5 mmol/L was calculated in both the losartan and placebo group. To identify the predictors of increased serum potassium at month 6, a multivariate logistic regression model was used. Baseline characteristics which showed an association with serum potassium ≥ 5.0 mmol/L (P<0.2) at univariate analysis were selected for the multivariate logistic model. The multivariate logistic model was adjusted for age, treatment assignment, serum potassium, diastolic blood pressure, estimated Glomerular Filtration Rate (eGFR), month 6 change in eGFR from baseline, urinary albumin:creatinine ratio (ACR), prescription of α-blockers, thiazide diuretics, loop diuretics, and hemoglobin. To assess the association between change in serum potassium from baseline to month 6 and renal outcomes, a multivariate Cox proportional hazard model was used. The linearity of baseline and follow-up continuous variables was assessed. If the linearity was not demonstrated, the variable was recoded as a categorical variable. In the final Cox model we adjusted for the following baseline variables: age, gender, race, treatment, eGFR, follow-up systolic blood pressure, diastolic blood pressure, ACR. We checked for an interaction between serum potassium levels at month 6 and eGFR. To ensure that our results are not affected by baseline renal function and other important predictors of renal outcomes, such as blood pressure and urinary albumin excretion we performed a sensitivity analysis in which we matched patients based on their propensity score of developing serum potassium ≥ 5.0 mmol/L. The propensity score was obtained by performing a logistic regression model with serum potassium ≥ 5.0 mmol/L as an outcome. The risk of renal outcomes was presented by hazard ratios (HR) with 95% confidence intervals (95% CI). Analyses were conducted with SPSS version 16.0 software.

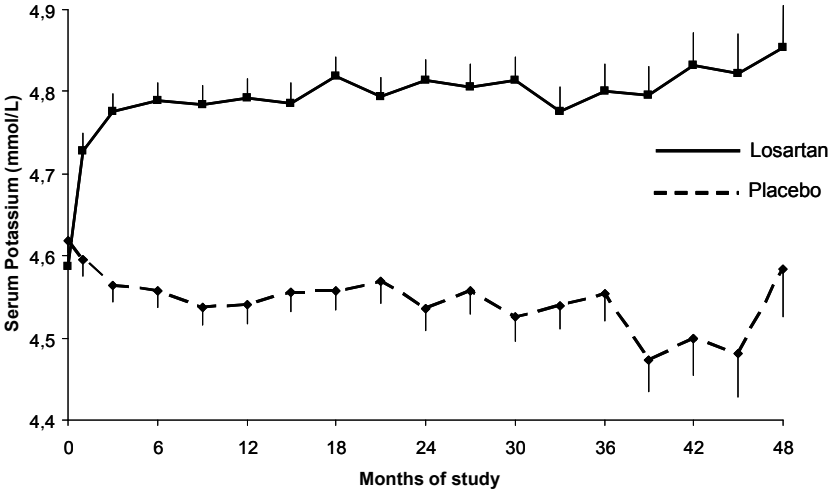
Results

Serum potassium over time and characteristics of the study population

In the whole population at month 6, 928 (69.4%) patients had a serum potassium <5.0 mmol/L (normal value) while 410 (30.6%) patients had month 6 serum potassium ≥5.0 mmol/L (Table 1). In the losartan group, mean potassium level significantly increased from 4.59 mmol/L at baseline to 4.79 mmol/L at month 6, and remained relatively stable during follow-up (Figure 1). In contrast, in the placebo group, the mean potassium level decreased gradually from 4.62 mmol/L at baseline to 4.56 mmol/L at month 6, and remained relatively stable thereafter.

Figure 1: Mean serum potassium level during follow-up among patients who were assigned to receive losartan or placebo

Bars represent standard errors.



The percentage of patients with month 6 serum potassium levels ≥ 5.0 mmol/L and ≥ 5.5 mmol/L increased from 167 (22.2%) at baseline to 259 (38.4%) and 22 (2.9%) at baseline to 73 (10.8%) at month 6 respectively in patients on losartan, while in those on placebo it decreased from 200 (26.2%) to 151 (22.8%) and from 35 (4.6%) to 34 (5.1%) respectively (Figure 2).

Patients with serum potassium levels ≥ 5.0 mmol/L at month 6 were more likely to have higher baseline serum potassium levels, higher ACR, lower diastolic blood pressure and lower hemoglobin levels compared to patients with serum potassium <5.0 mmol/L (Table 1). The use of losartan was more common while the use of thiazide diuretics and α-blockers was less common in patients with serum potassium ≥5.0 mmol/L.

Table 1: Baseline and month 6 characteristics of the whole population[a]

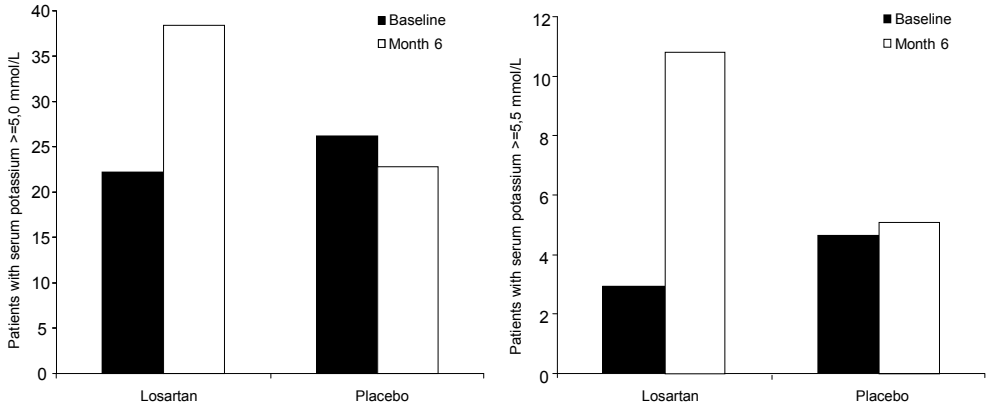
Baseline Characteristics	Serum potassium at month 6	
	<5.0 mmol/L (n=928)	≥5.0 mmol/L (n=410)
Age, yrs	60.0 (7.6)	60.4 (7.1)
Male, n (%)	593 (63.9)	248 (60.5)
Race, n (%)		
White	449 (48.4)	198 (48.3)†
Black	161 (17.3)	40 (9.8)
Hispanic	144 (15.5)	98 (23.9)
Asian	163 (17.6)	68 (16.6)
Other	11 (1.2)	6 (1.5)
Systolic BP, mmHg	152.1 (19.2)	153.2 (19.7)
Diastolic BP, mmHg	82.9 (10.5)	81.3 (10.1)[b]
Urinary ACR, mg/mmol, median (IQR)	129 (59-263)	155 (76-327)[b]
Serum creatinine, mg/dl	1.8 (0.5)	2.0 (0.5)[b]
eGFR, ml/min/1.73m^2	40.9 (12.2)	37.0 (11.9)[b]
HbA1C, %	8.4 (1.6)	8.4 (1.6)
Hemoglobin, mg/dL	12.7 (1.8)	12.1 (1.8)[b]
Serum potassium, mmol/L	4.5 (0.5)	4.9 (0.4)[b]
Serum potassium ≥ 5.0 mmol/L, n (%)	140 (15.1)	187 (45.6)[b]
Treatment, n (%)		
Losartan	416(44.8)	259 (63.2) [b]
Thiazide diuretics	161 (17.3)	45 (11.0)[b]
K-sparing diuretics	24 (2.6)	7 (1.7)
Loop diuretics	426 (45.9)	177 (43.2)
Calcium channel blocker	679 (73.2)	291 (71.0)
α-blockers	239 (25.8)	81 (19.8)[b]
β-blockers	173 (18.6)	76 (18.5)
Month 6 characteristics		
Systolic BP, mmHg	149.3 (19.8)	150.1 (20.4)
Diastolic BP, mmHg	81.2 (10.7)	79.3 (10.4)[b]
Urinary ACR, mg/mmol, median (IQR)	124 (46-249)	139 (51-291)[b]
eGFR, ml/min/1.73m^2	38.1 (14.2)	32.9 (13.2) [b]

Abbreviations are: BP – blood pressure, ACR – albumin-creatinine ratio:

[a]Data are presented as means (SD) unless otherwise indicated

[b]P<0.05 between patients with K ≥ 5.0 mmol/L and those with K <5.0 mmol/L at month 6

Figure 2: Proportion of patients with serum potassium ≥ 5.0 mmol/L and ≥ 5.5 mmol/L at baseline and month 6 among patients assigned to losartan and placebo



Predictors of incident serum potassium ≥5.0 mmol/L at month 6

In testing in multivariate analysis which baseline parameters are related to increased serum potassium ≥5.0 mmol/L at month 6, we found that the strongest baseline predictors were losartan therapy (OR 2.81; 95%CI 2.03-3.89) and serum potassium (OR 2.26; 95%CI 1.51-3.37). In contrast, a decreased eGFR was associated with an increased risk of high serum potassium. (Table 2).

Table 2: Baseline multivariate predictors of incident drug induced serum potassium ≥ 5.0 mmol/L at month 6

Risk marker	Odds Ratio (95%CI)	$\chi 2$	P value
Losartan treatment	2.80 (2.02 – 3.88)	38.3	<0.001
Serum potassium, mmol/L	2.30 (1.53 – 3.44)	26.2	<0.001
eGFR, ml/min/1.73m^2	0.98 (0.97 – 0.99)	6.2	0.013
α-blocker use	0.68 (0.46 – 1.01)	3.6	0.058
Loop diuretic use	0.75 (0.53 – 1.04)	3.0	0.085
Month 6 change eGFR, ml/min/1.73m^2	1.02 (0.99 – 1.05)	2.9	0.086
Age, yrs	1.02 (0.99 – 1.04)	2.2	0.141
Hemoglobin, mg/dL	0.94 (0.85 – 1.04)	1.5	0.228
Diastolic blood pressure, mmHg	0.99 (0.98 – 1.01)	1.3	0.252
Log transformed ACR, log unit mg/g	1.06 (0.89 – 1.26)	0.4	0.541
Thiazide use	0.82 (0.53 – 1.27)	0.8	0.372

Presented risk markers were selected for multivariate analysis if an association with serum potassium ≥ 5.0 mmol/L was demonstrated in univariate analyses. Risk markers are ordered according the $\chi 2$ values.

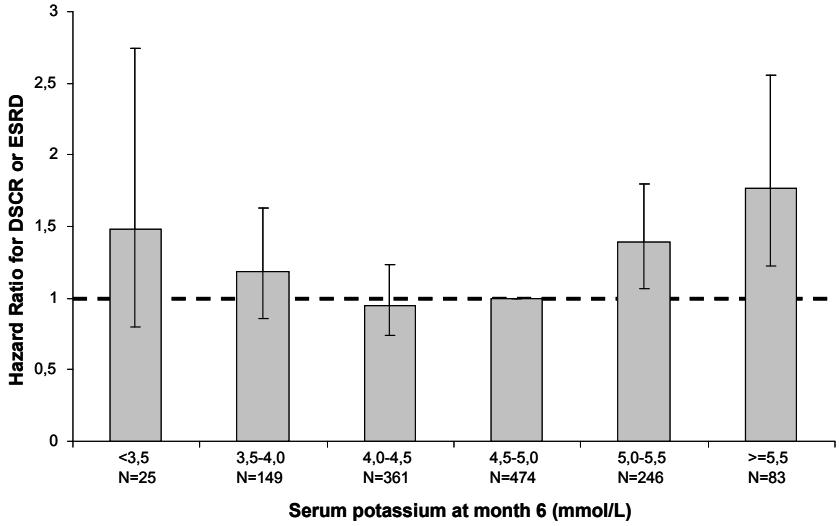
Month 6 serum potassium and renal outcomes

Serum potassium level was associated with a higher risk of the composite renal outcome of DSCR or ESRD. As observed in Figure 3A, the risk already started to significantly increase from serum potassium ≥5.0 mmol/L and further increased at serum potassium ≥5.5 mmol/L during follow-up (HR 1.39; 95% CI 1.07-1.80 and HR 1.77; 95% CI 1.22-2.56 respectively). However several other factors also explained the progressive loss of renal function such as age, eGFR, and ACR. The most important question was therefore whether the progressive nature of renal end points in patients with serum potassium ≥ 5.0 mmol/L is independent of other factors, and most importantly, by the prevailing renal function, since patients with low eGFR are more prone to develop high serum potassium levels. After adjustment for other risk factors, month 6 serum potassium ≥ 5.0 mmol/L was associated with a 22% increased risk for developing adverse renal outcomes (HR 1.22; 95% CI 1.00-1.50). Further analysis revealed that this increased risk was merely attributed to patients with persistent drug induced serum potassium ≥ 5.0 mmol/L both at month 6 and month 9 (HR 1.56; 95%CI 1.09-2.21) (Table 3).

Figure 3: Month 6 serum potassium level (3A) and mean serum potassium (3B) and the risk for the composite renal endpoint (DSCR or ESRD)

Bars represent 95% Confidence Intervals

3A



3B

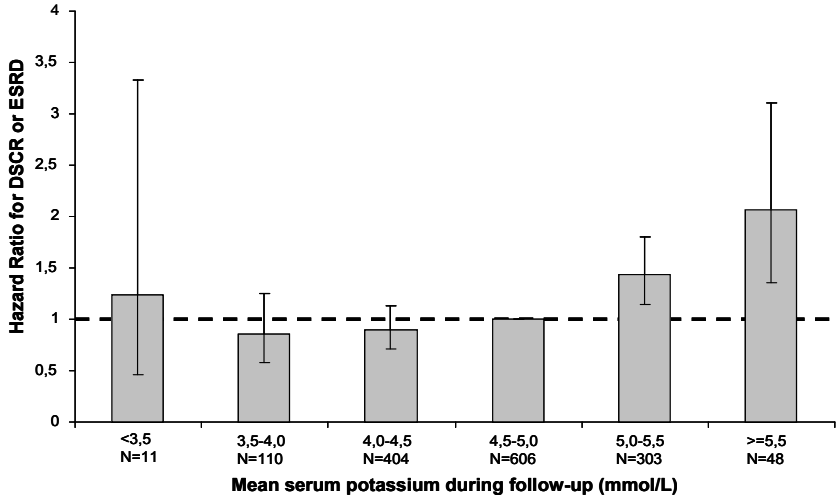


Table 3: Persistent and single elevated serum potassium ≥ 5.0 mmol/L and their association with the risk for doubling of serum creatinine or ESRD[a]

Risk Factors	HR (95%CI)	$\chi 2$	P value
Drug induced persistent[b] serum potassium ≥5.0 mmol/L (month 6 and 9)	1.54 (1.07 – 2.22)	5.43	0.020
Single[c] elevated serum potassium ≥5.0 mmol/L (month 6 or 9)	1.26 (0.93 – 1.70)	2.15	0.142
Age	0.97 (0.96 – 1.00)	2.34	0.126
Race (reference: White)			
Black	2.13 (146 – 3.10)	15.57	<0.001
Asian	1.42 (1.01 – 1.99)	3.99	0.046
Other	1.62 (1.18 – 2.21)	9.04	0.003
eGFR	0.96 (0.95 – 0.97)	46.63	<0.001
Systolic blood pressure	1.01 (1.00 – 1.02)	7.15	0.008
Diastolic blood pressure	0.99 (0.98 – 1.01)	1.42	0.233
Albumin:creatinine ratio	3.75 (3.12 – 4.51)	196.83	<0.001
Treatment (losartan/placebo)[d]	0.92 (0.71 – 1.20)	0.38	0.538

[a] Essentially similar results were obtained for the individual components of the endpoint (data not shown).

[b] Persistent elevated serum potassium defined as drug induced serum potassium ≥5.0 mmol/l at month 6 and 9.

[c] Single elevated measurement defined as serum potassium ≥5.0 mmol/l at month 6 or 9.

[d] There was no interaction between treatment groups and high potassium at month 6 and 9 (p=0.284) indicating that the association between high potassium and renal outcome are consistent across both treatment groups.

In the second approach we assessed the association between the mean serum potassium level during follow-up with renal outcomes. The relationship between the mean serum potassium level during follow-up displayed a similar pattern with renal outcomes as the month 6 serum potassium level (Figure 3B). After controlling for potential confounders, the analyses revealed that patients who achieved a mean serum potassium ≥ 5.0 mmol/L during follow-up had a 43% higher risk of the composite endpoint of DSCR or ESRD (HR 1.36; 95% CI 1.11-1.67).

The sensitivity analysis in 712 patients matched per propensity score showed similar detrimental effects of increased serum potassium ≥ 5.0 mmol/L on renal outcomes (HR 1.32; 95%CI 1.03-1.70). There was no heterogeneity between increased serum potassium and eGFR (p=0.132).

Effect of serum potassium ≥ 5.0 mmol/L on the renoprotection induced by losartan

To examine to what extent the increase in potassium influences the renoprotective effect afforded by losartan, we analyzed the impact of an increase in serum potassium on the losartan treatment effect. When the treatment effect on losartan was adjusted for the residual potassium level (last potassium level measured prior to the renal endpoint), the treatment effect of losartan on the DSCR or ESRD endpoint increased from 21% (6%–34%) to 35% (20%–48%). This suggests that the effect of losartan on serum potassium offsets the renoprotective effect of losartan.

Discussion

In this study, we showed that treatment with losartan increased the serum potassium concentration. We furthermore demonstrated that the occurrence of high serum potassium levels increased the risk of adverse renal outcomes and counteract the beneficial renoprotective effects of losartan. The increase in the renal risk appeared to be independent of other important renal risk factors, such as blood pressure, eGFR and ACR. Thus, although the RENAAL trial has clearly shown that losartan is a renoprotective drug, under this protection a renal damaging effect is hiding in those patients where losartan induces high serum potassium levels.

The effects of the ARB losartan on serum potassium are in line with other studies. In patients with diabetes, either addition or administration of an ARB increases the incidence of hyperkalemia, independent of renal function.(8) Also, in patients with heart failure, addition of an ARB or aldosterone antagonist to baseline RAAS inhibitor therapy increases the risk of hyperkalemia. (9, 12) In contrast, in non-diabetic patients addition of RAAS-inhibitors poses a minimal risk of hyperkalemia as long as renal function is relatively preserved.(13-17) It appears that the risk of hyperkalemia is particularly elevated in patients with underlying predisposing disorders, such as diabetes and renal insufficiency, and in patients who receive combined RAAS therapy.

The mechanism via which ARB treatment induces elevations in serum potassium levels has already been described.(6) In short, potassium excretion is mainly regulated by serum aldosterone and sodium delivery to the distal nephron. Blocking the effects of Angiotensin II by RAAS-inhibitors decreases aldosterone production and consequently induces hyperkalemia. Patients with diabetes are particularly susceptible to the hyperkalemic effects of RAAS-inhibitors as their RAAS-activity is already suppressed. Several factors may account for this, such as an impaired conversion of pro-renin to renin(18) or volume expansion with subsequent increase in circulating atrial natriuretic peptide levels and suppression of plasma renin activity.(19)

In previous studies no data are available on the effect of high serum potassium levels on

renal outcomes. Our study showed for the first time that increased serum potassium concentrations ≥ 5.0 mmol/L is associated with a clearly increased risk of DSCR or ESRD, independent of renal function and other important predictors of renal outcomes. The pathophysiological mechanism whereby increased serum potassium levels affect renal outcomes is not well known. It is likely that individuals with persistent drug induced hyperkalemia are resistant against the kaliuretic effects of aldosterone. It has indeed been shown that the trans-tubular potassium gradient, as measure for aldosterone bioactivity with respect to its kaliuretic response, is decreased in individuals with drug induced hyperkalemia despite increased plasma aldosterone levels.(20) Consequently, these individuals are continuously exposed to the deleterious effects of aldosterone on renal tissue. Another potential mechanism could be that a vicious cycle exits between renal function and potassium levels which usually takes place in disorders that affect both tubular dysfunction and release of renin. On the one hand, a decrease in renal perfusion and the start of tubulointerstitial damage may impair renal potassium excretion, even though renal function is only mildly depressed. This may lead to an imbalance in renal potassium/sodium handling which may further damage the tubules, thereby subsequently contributing to a further decline in renal function.(7)

Several reports have drawn attention to spurious hyperkalemia (pseudohyperkalemia) as a common problem in clinical care.(21, 22) The reasons for spurious hyperkalemia are multiple, such as inappropriate phlebotomy technique (e.g. requesting patient to fist clench to facilitate venesection), improper sample storage (i.e. cold storage or too long storage causing deterioration of the sample specimen) or contamination with anticoagulant from another sample (EDTA contamination).(21, 22) As it is unlikely that subjects with a single erroneous potassium measurement are at increased risk, we classified patients in those who had persistent high serum potassium levels at month 6 and 9. As expected, the increased risk for renal outcomes was particularly marked in individuals with high serum potassium at both visit 6 and 9. This implies that elevation in serum potassium level needs particular attention and appropriate management if it is confirmed at a follow-up visit. In addition, our data on the relationship between the mean potassium level during follow-up, which reflects the exposure to a high serum potassium load during a definitive period of time more accurately than a single value, and renal outcomes displayed a similar association between increased serum potassium and adverse renal outcomes. These results are in clear contrast to a recent report from Weir et.al. who suggested that the changes in serum potassium concentration observed during RAAS-therapy are unlikely to be clinically significant. (23) We recommend not down playing modest changes in serum potassium as they independently indicate increased risk for renal outcomes in the long-term.

Hyperkalemia is usually defined by a serum potassium concentration ≥5.5 mmol/L. Our results demonstrated a distinct risk of adverse renal events in not only patients with serum potassium concentration ≥5.5 mmol/L, but also in patients with potassium concentrations ≥5.0 mmol/L during follow-up and at month 6. These results have important consequences for clinical practice as they indicate that the risk for renal events already starts to increase within ranges that are currently considered to be normal. Particular caution is needed when prescribing a second RAAS-agent as the combination of RAAS-inhibitors may lead to even higher serum potassium levels.(24, 25) In patients with high potassium levels at start of ARB therapy, it may be initiated with a low dose, and increased to a higher dose if serum potassium levels do not increase above a therapeutic threshold.

Would improved management of high serum potassium levels lead to better renal outcomes associated with RAAS blockade? Our study does not directly answer this question. However, when we adjusted the treatment effects by the residual serum potassium levels measured prior to the renal endpoint the renoprotective effects associated with losartan use markedly improved. It is therefore tempting to speculate that management of high serum potassium levels improves the renoprotective effects of losartan. Further prospective randomized controlled trials are needed to confirm this finding.

Our study has some limitations. First, this is a post-hoc analysis, and as such may be subject to confounding. To control for confounding we adjusted for a wide range of clinical variables, both at baseline and follow-up. It is nevertheless possible that residual confounding remained even in our multivariate adjusted analysis. Also, we performed two additional sensitivity analyses matching patients on their eGFR and propensity score to ensure that renal events are independent of important predictors of increased serum potassium. Second, although the RENAAL trial included a broad range of patients with type 2 diabetes and nephropathy, the findings cannot be extrapolated to other populations.

In conclusion, in this study we found that treatment with the ARB losartan is associated with a high risk of serum potassium level elevation in patients with type 2 diabetes and nephropathy. This elevated serum potassium level is in turn associated with an increased risk of renal outcomes and offsets the renoprotective effects of losartan. Whether additional management of elevated serum potassium would further increase the renal protective properties of losartan is an important clinical question.

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CHAPTER 4b

High serum potassium levels after losartan can reflect more severe renal disease. Reply to Gonçalves AR, El Nahas AM (Letter)

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Journal

Diabetologia. 2011 Nov;54(11):2965-7.

Original letter from Gonçalves and El Nahas

Diabetologia (2011) 54:2963–2964
DOI 10.1007/s00125-011-2220-7

LETTER

High serum potassium levels after using losartan can reflect more severe renal disease

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Received: 9 February 2011 / Accepted: 25 May 2011 / Published online: 18 June 2011

Keywords Hyperkalaemia · Inhibitors of the renin–angiotensin system · Renal disease progression · Tubulointerstitial damage

Abbreviations

RAS	Renin–angiotensin system
RENAAL	Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan
TI	Tubulointerstitial

To the Editor: We read with great interest the paper by Miao et al. [1] showing in a post hoc analysis of the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) study [2] that increased serum potassium levels affect renal outcomes. The authors' observations are intriguing as they show that not all the patients in the study benefited from using inhibitors of the renin–angiotensin system (RAS); in fact, some may have suffered adverse events, including an acceleration of renal disease progression, most notably in those with hyperkalaemia. Miao and colleagues argue that the changes in potassium levels are dependent on losartan therapy, but its effect on risk for renal events is independent of the use of losartan (Table 3 in Miao et al. [1]). This is intriguing, as the percentage of patients on potassium-sparing diuretics is quite small and most of the other antihypertensive medications are not commonly associated with hyperkalaemia.

Miao et al. state that changes in potassium levels could reflect the severity of tubulointerstitial (TI) damage. This is supported by the observation that the high potassium group also had lower baseline haemoglobin levels, which is compatible with more severe interstitial damage. It is well established that the hyporeninaemic hypoaldosteronism syndrome and low levels of erythropoietin are associated, probably reflecting damage to the juxtaglomerular apparatus and interstitial cells, respectively [3].

Diabetic nephropathy is a heterogeneous disease in patients with type 2 diabetes [4]. Proteinuria does not always predict progression [4]. It would be interesting to know whether in the RENAAL patients there was a relationship between the changes in the albumin to creatinine ratio and potassium levels. There are different histological patterns in diabetic kidney disease: most patients have typical glomerular basement membrane thickening and mesangial expansion, while some have significant glomerulosclerosis and others have severe TI scarring. In fact, it is TI scarring that correlates best with the severity of renal impairment and predicts the progression of diabetic kidney disease [5]. It comes as no surprise, at least

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to us, therefore, that hyperkalaemia as a possible indicator of more severe TI damage is associated with faster progression of diabetic nephropathy.

Should we then continue the use of inhibitors of RAS in hyperkalaemic patients with potentially progressive diabetic kidney disease? The continued use of inhibitors of RAS in such patients is associated with worse outcomes [6, 7].

With this in mind, we disagree with the authors' conclusion that increased serum potassium levels dampen the renoprotective effect of losartan. We believe that it is more likely that those patients with high serum potassium levels and increased risk of adverse renal outcomes are either those who have received higher doses of inhibitors of RAS, have increased sensitivity to a similar dose, or have more severe TI disease, and hence faster progression of their underlying nephropathy.

Acknowledgements Both authors have contributed equally to the conception, writing and final approval of this letter.

Duality of interest statement The authors declare that there is no duality of interest associated with this manuscript.

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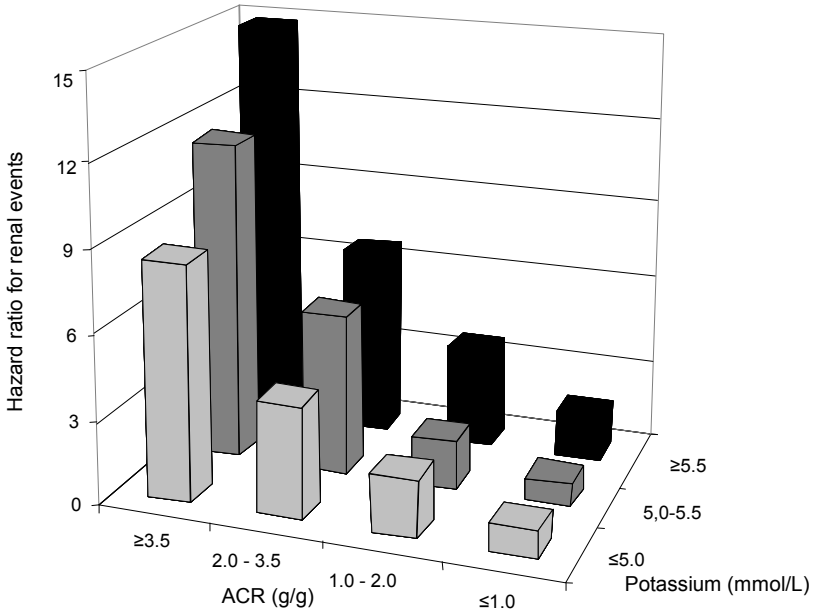
To the Editor: Gonçalves and El Nahas (1) raise interesting comments on our publication describing how blockade of the angiotensin I receptor with losartan increases serum potassium which is, in turn, associated with an increased risk of renal outcomes in patients with diabetes and nephropathy.(2) In their letter, Gonçalves and El Nahas suggest that those patients who developed hyperkalaemia were more likely to have pre-existing glomerular or tubulointerstitial damage, which could have accounted for the faster progression of renal disease. Although this could, to a certain extent, hold true, we believe that such a theory does not explain our findings.

We performed various analyses to ensure that the relationship between increased serum potassium and renal outcome was independent of the severity of the underlying renal disease. First, we corrected for various renal risk markers, most notably the baseline and change in estimated glomerular filtration rate. Second, we conducted a pair-matched analysis selecting patients with similar characteristics who did and did not develop hyperkalaemia. Both analyses consistently showed that participants in whom serum potassium levels rose had a higher risk of renal disease. We therefore consider it unlikely that the greater degree of pre-existing renal injury in individuals who developed high serum potassium levels is the main factor responsible for their higher renal risk. Nevertheless, since neither glomerular nor tubulointerstitial damage was measured in the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) trial, we cannot completely rule out the possibility that individuals who developed hyperkalaemia also had pre-existing renal damage.

Further indirect evidence to support our hypothesis that the increase in serum potassium is associated with renal outcome stems from an additional analysis showing that adjustment of the losartan treatment effect for the residual potassium level (the serum potassium level during treatment) improves the overall renoprotective response to losartan. These data suggest that the increase in serum potassium during losartan therapy attenuates its long-term renoprotective effect and imply that improved management of hyperkalaemia may result in a better renal outcome. As mentioned by Gonçalves and El Nahas,(1) it could still be possible that individuals who experienced high serum potassium levels received a higher dose of losartan. This hypothesis is, however, less likely because the dose of losartan was titrated towards 100 mg/day in almost all patients. Gonçalves and El Nahas also suggested that those patients who experienced high serum potassium levels could have been more sensitive to a similar dose of losartan.(1) Nevertheless, this does not negate the fact that the development of high potassium levels appears to offset the renoprotective effect of losartan.

Given that both the short-term responses to losartan in terms of serum potassium levels and albuminuria are associated with renal outcomes, either positively (albuminuria) or negatively (potassium), Gonçalves and El Nahas (1) highlight the importance of evaluating the relationship between both of these responses within individual patients. Such question is of clinical relevance since these opposing responses in individual patients may offer important insight into their ultimate renal outcome. In this regard, we performed additional analyses to investigate possible correlations between responses in serum potassium levels and albuminuria within each individual patient in the RENAAL trial. The majority of patients (52.6%) showed a decrease in albuminuria and increase in serum potassium levels during treatment with losartan. However, a substantial number of patients experienced either reductions in both albuminuria and serum potassium levels (17.7%), an increase in albuminuria and a decrease in serum potassium levels (9.6%) or an increase in both variables (20.0%). These data illustrate that the change in two variables in response to a single drug varies within an individual. The group of patients in whom both albuminuria and serum potassium rose, and thus had high residual albuminuria and high serum potassium levels during losartan therapy, had a very high risk of renal events, as shown in Fig. 1. However, our findings are based on a post hoc analysis, and a prospective evaluation is needed to prove that manipulation of serum potassium levels during angiotensin receptor blocker (ARB) therapy may result in improved renal outcomes. Nevertheless, these data clearly support the need for monitoring and optimising the response of serum potassium levels and albuminuria in individual patients to improve the long-term renal protective effects of ARBs.

Figure 1: Risk for renal events by mean levels of albuminuria (ACR, mg/mmol) and serum potassium (mmol/l) during follow-up. The figure shows that both the residual albuminuria and serum potassium determine the renal outcome. For conversion of ACR to mg/g multiply by 8.84.



Based on our observation that losartan treatment is associated with an increase in serum potassium levels, which increases renal risk, should we reconsider the continued use of inhibitors of the renin–angiotensin–aldosterone system (RAAS)? Gonçalves and El Nahas (1) indeed questioned the use of RAAS inhibitors and refer to the recent Ongoing Telmisartan Alone and in Combination with Ramipril Trial (ONTARGET), which showed increases in renal risk and hyperkalaemic events associated with combined angiotensin-converting enzyme inhibitor (ACEI) and ARB therapy compared with the single use of these agents.(3) However, given the proven renal and cardiovascular protective effects of ACEIs and ARBs in various populations (including those with diabetes and nephropathy),(4, 5) we strongly discourage the discontinuation of these agents. Instead, strategies to optimise the effect of a drug on the good surrogates, such as blood pressure and albuminuria, and to minimise its effect on the bad surrogates, such as serum potassium, are recommended. This approach may attenuate the high renal risk of the growing population of patients with diabetes and nephropathy.

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CHAPTER 5

The importance of short-term off-target effects in estimating the long-term renal and cardiovascular protection of Angiotensin Receptor Blockers

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Abstract

Background: Antihypertensive drugs are developed and registered on the basis of blood pressure lowering efficacy combined with safety. However, the long term use has the purpose of preventing cardiovascular (CV) morbidity and mortality. Interestingly, antihypertensive drugs have multiple off-target effects that may contribute to its efficacy on CV outcomes. The aim of the present analysis was to assess whether a multiple parameter risk response outcome (PRO) score, incorporating the drug's short term on-target and off-target effects, better predicts the ultimate renal/CV protection than changes in single on-target or off-target effects.

Method: Data were used from subjects with type 2 diabetes and nephropathy participating in the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) or Irbesartan Diabetic Nephropathy Trial (IDNT) trials. A PRO score was developed by multivariate Cox analysis in the placebo arm of the RENAAL trial and was then applied to the baseline and month-6 measurements of the ARB treatment arm of the RENAAL trial to predict renal or CV risk. The net risk difference at these time-points after correction for placebo effects indicated the estimated long-term drug effect. Subsequently, the obtained PRO score was validated in the dataset of the IDNT trial.

Results: Changes in single risk markers (e.g. blood pressure) predicted relative risk reductions (RRR) significantly different from the actual observed RRR, both for renal (5.7%, vs. 21.8%, respectively), and CV outcomes (3.0%, vs. 9.2%, respectively). However, the PRO score accurately predicted both renal ($RRR_{predicted}$: 30.1 (95% Confidence Interval 10.8 to 49.5) vs. 21.8 (6.5 to 34.5); p=0.44) and CV treatment effect ($RRR_{predicted}$: 9.4 (1.9 to 17.0) vs. 9.2 (-7.6 to 23.6), p=0.98). Validation of the PRO score in another diabetes trial (IDNT) accurately predicted the renal ($RRR_{predicted}$: 26.6 (14.3 to 38.9) vs. 26.0 (6.4 to 41.5) p=0.95) and CV treatment effect ($RRR_{predicted}$: 7.9 (1.3 to 14.5) vs. 11.9 (-8.4 to 28.5) p=0.67).

Interpretation: A PRO score based on month-6 changes in on-target and off-target risk markers performs better in estimating ARB effects on hard renal and CV outcomes than any score based on changes in single on-target or off-target risk markers.

Introduction

The ultimate public health goal of antihypertensive therapy is to reduce the risk of renal and cardiovascular (CV) morbidity and mortality.[1] Antihypertensive drugs are however not registered based on their efficacy to reduce the risk of renal or CV events, but are developed and registered based on their blood pressure lowering capacity. To this end, the effect of the drug on blood pressure is established in short-term studies and is subsequently used to estimate the potential long-term renal or CV protective effect. This process assumes firstly that the drug effect on blood pressure (the on-target risk factor) is directly associated with a reduction in the risk of renal or CV complications, and secondly, that the drug does not influence other risk factors (off-target risk factors) that influence renal or CV events. The latter assumption has been challenged.

The Losartan Intervention For Endpoint reduction (LIFE) trial showed that the Angiotensin Receptor Blocker (ARB) losartan exerted equal blood pressure lowering effects as the β-blocker atenolol but conferred superior CV protective effects.[2] The Irbesartan Diabetic Nephropathy Trial (IDNT) showed that the ARB irbesartan conferred additional renal protective effects compared to the Calcium Channel Blocker amlodipine at equal blood pressure control.[3] Finally, the Renoprotection of Optimal Anti-proteinuric Dose (ROAD) trial showed that a supra-maximal dose of losartan improves the anti-albuminuric response and conferred markedly more renoprotection at similar blood pressure control achieved with losartan at the maximally recommended blood pressure dose.[4] These studies suggest that antihypertensive drugs, in these examples ARBs, exert additional beneficial effects on renal or CV risk factors, so called off-target effects, which contribute to the ultimate long-term effect of the drug. At the other hand, several studies have shown that antihypertensive drugs may induce changes in risk factors, such as serum potassium, which in fact increase the risk for renal/CV outcome, thus counteracting the beneficial effects of these drugs.[5, 6] This implies that only focusing on blood pressure, the on-target risk factor, may result in a misleading impression of the drug's protective efficacy. We hypothesize that knowing the short term effect of an antihypertensive on all renal/CV risk markers would allow the composition of a response score that would better predict the long term effect of such a drug on the ultimate renal/CV outcome. This may have major consequences for drug development, drug registration, and individual patient care and highlights the necessity to identify those off-target effects.

The aim of the present analysis was firstly to identify off-target effects of an ARB and assess the impact of the off-target effect on the ARB's renal/CV efficacy. Secondly, we aimed to construct a multiple parameter risk response outcome (PRO) score based on the short-term (6 months) on-target and off-target effects of ARBs in order to estimate the effect of the drug on long term renal/

CV morbidity and mortality. Thirdly, we compared the accuracy of ARB efficacy estimates based on the multiple PRO score with scores based on single on-target or off-target risk markers. Finally, we validated the accuracy of the PRO score in a separate different trial dataset.

Methods

Study design

Data were used from the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) and IDNT trials. The rationale, study design and outcomes for these trials have been previously published and were almost exactly similar.[3, 7-9] In brief, the overall aim of the trials was to assess the impact of an ARB on hard renal (primary endpoint) and CV outcomes (secondary endpoint) by testing losartan 100 mg/day vs. placebo in the RENAAL trial and irbesartan 300 mg/day vs. placebo in the IDNT trial. The IDNT trial also included a Calcium Channel Blocker arm which was excluded from the present analysis. Inclusion criteria for both trials were presence of type 2 diabetes, nephropathy, and age between 30 and 70 years. Subjects with insulin dependent diabetes or renal disease not related to diabetes were excluded in both trials. All subjects gave written informed consent. Both trials were approved by local medical ethics committees and conducted according to the guidelines of the declaration of Helsinki.

Measurements

In both RENAAL and IDNT, renal and CV risk markers were measured at baseline and at 6 months intervals thereafter. All risk markers collected at baseline and month 6 were used to create the PRO-score. We selected all measured risk markers at month 6 because we did not know a priori which risk markers would change during ARB therapy and secondly to exclude any potential bias as a result of risk marker selection. Changes in on-target and off-target risk markers after ARB treatment were calculated as the difference between the baseline and the 6-month value. 6-month values were chosen because most parameters were available at 6-month and ARB treatment effects were considered fully present. Since total cholesterol, hemoglobin, serum albumin, calcium, and phosphate were not measured at month 6 in the RENAAL trial, 12-month values were used.

Renal and cardiovascular outcomes

The primary outcome for the present analysis was defined as a composite of a confirmed doubling of serum creatinine from baseline (DSCR) or end-stage renal disease (ESRD). The latter was defined as chronic dialysis or renal transplantation. The secondary CV outcome was another

endpoint for the present study which was defined in both trials as the composite of myocardial infarction, stroke, hospitalization for heart failure, revascularization procedures or death related to CV disease. All renal and CV events were adjudicated by an independent blinded committee using rigorous definitions.

Model development

Parameter risk response scores were developed by estimating the risk relation between single or multiple risk markers and renal or CV outcomes in the placebo group of the RENAAL trial. The single and multiple parameter risk response outcome scores were subsequently applied to the baseline and 6-month measurements of the ARB treatment arm to predict renal or CV risk at both time points. The difference in the estimated risk at these time-points, adjusted for the difference in estimated risk in the placebo arm, indicates the long-term renal or CV risk change conferred by ARB treatment. To test the validity of this approach, the single and multiple parameter risk response outcome scores were compared with the actual observed renal or CV outcomes of the trials. Any model shows too optimistic performance from the dataset from which it is developed. The risk response scores were externally validated by developing the scores in the RENAAL trial and testing them in the IDNT trial.

Model evaluation

The methodology to develop the PRO score assumes that the association between risk markers at baseline and renal or CV events in the placebo group is similar as the association between single or multiple risk markers at 6-month and renal or CV events during ARB therapy. To verify the validity of this assumption, we determined whether 6 months ARB treatment modified the association between risk markers and renal or CV events. We detected no interaction between any risk marker and ARB treatment for the renal or CV outcome as tabulated in table 1. This indicates that ARB treatment did not modify the association between single or multiple risk markers and renal or CV events. Imputation of missing data yielded essentially similar results as the main analyses.

Table 1: Absence of significant interaction between risk markers at baseline in the placebo group and risk markers at month 6 in the ARB treatment group with renal or CV outcomes in the RENAAL trial. Similar results were obtained in the IDNT trial (data not shown)

	Renal outcomes		CV outcomes	
Baseline variables	β-coefficient	P-value Interaction	β-coefficient	P-value interaction
systolic BP	0.01		0.00	
treatment†	-0.31		-1.52	
systolic BP*treatment	0.00	0.94	0.01	0.13
log UACR	1.34		0.05	
treatment	1.79		-1.12	
log UACR* treatment	-0.26	0.35	0.18	0.21
potassium	-0.01		-0.05	
treatment	-1.88		0.62	
potassium* treatment	0.36	0.30	-0.11	0.69
hemoglobin	-0.33		-0.08	
treatment	-1.03		-0.77	
hemoglobin* treatment	0.05	0.63	0.08	0.28
uric acid	-0.05		-0.01	
treatment	-0.56		0.01	
uric acid* treatment	0.05	0.67	0.02	0.81
HbA1c	-0.03		0.03	
treatment	-0.74		-0.27	
HbA1c* treatment	0.06	0.66	0.05	0.58
total cholesterol	0.01		0.00	
treatment	0.00		0.01	
total cholesterol* treatment	0.00	0.76	0.00	0.84
BMI	-0.03		-0.01	
treatment	-0.30		-0.59	
BMI* treatment	0.00	0.91	0.03	0.28
serum albumin	-1.94		-0.05	
treatment	-0.75		1.81	
serum albumin* treatment	0.09	0.86	-0.45	0.19
calcium	-1.21		-0.35	
treatment	-1.16		-0.71	
calcium* treatment	0.09	0.82	0.08	0.76
phosphate	0.40		0.20	
treatment	-1.18		0.84	
phosphate* treatment	0.23	0.47	-0.19	0.37

†Treatment variable indicates whether patients received losartan or placebo

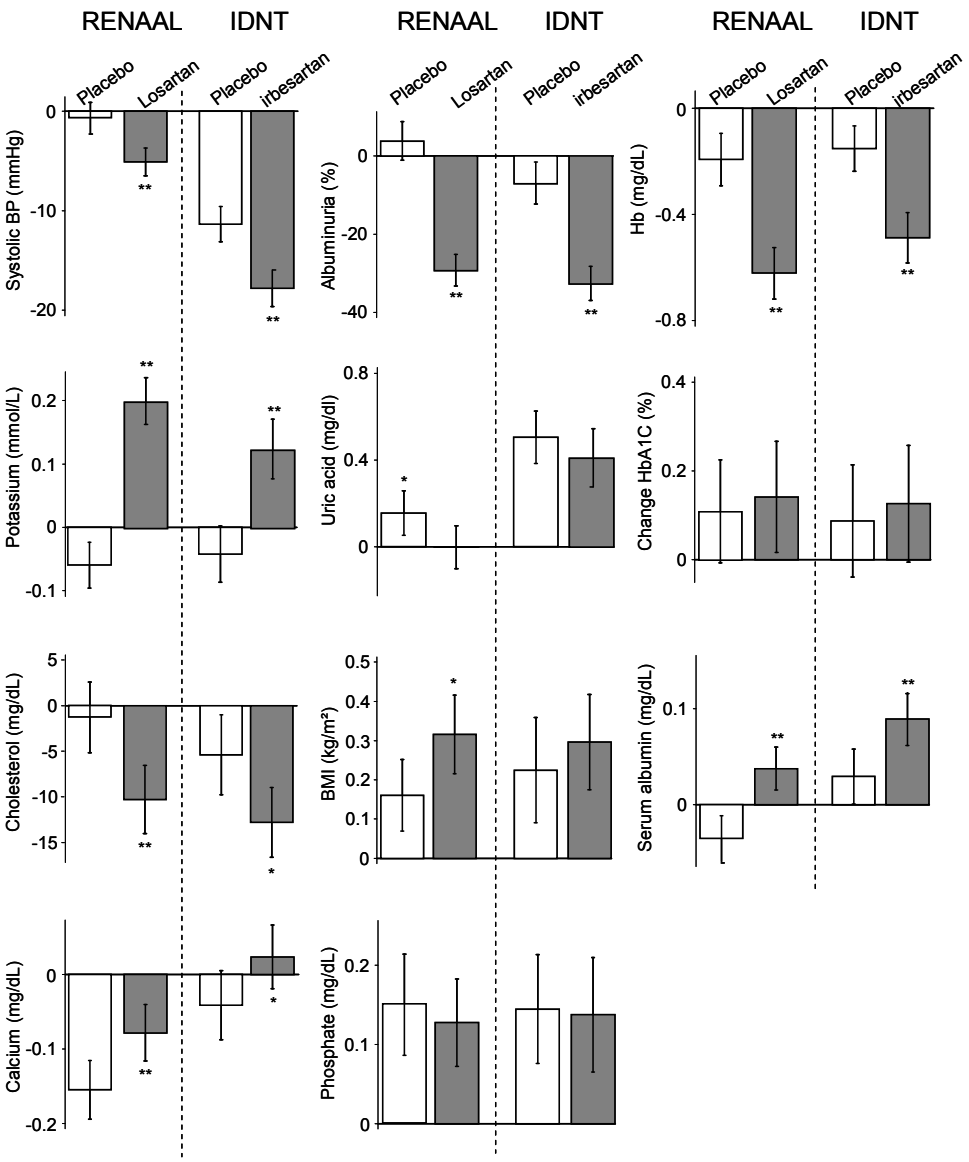
Statistical analysis

Mean and standard deviation were provided for 6-month changes in risk markers and statistical significance for the between group difference was determined based on a two sided t-test. Univariate and multivariate Cox proportional hazard regression was used to determine the relationship between baseline risk markers in the placebo treatment arm and renal or CV outcome. For subjects who experienced more than one renal or CV event during follow-up, survival time to the first relevant endpoint was used in each analysis. Participants were censored at their date of death or, for those still alive at the end of follow-up, the date of their last clinic visit before the termination of the trials. The multivariate Cox analysis included the following risk markers, systolic blood pressure, urinary albumin:creatinine ratio (UACR), potassium, hemoglobin, uric acid, HbA1c, total cholesterol, Body Mass Index, calcium, phosphate, and albumin. Bootstrap methods were applied, repeating the entire modeling with 1000 independent bootstrap samples, to include the variability of the regression coefficients of single and multiple risk scores and renal or CV outcomes. To determine the actual observed effect of losartan or irbesartan on renal/CV outcomes, the dichotomous treatment variable was used in a Cox regression model and the relative risk reduction was calculated as (1- hazard ratio) multiplied by 100%. Bootstrap methods, based on 1000 replications, were also used to derive the 95% confidence interval of the difference between the actual observed and predicted treatment effect. The difference between the predicted and observed treatment effect was tested by means of two sided t-tests. We verified normal distribution of the predicted treatment effect and performed log-transformation if required. Time-dependent Cox regression analysis was used to assess the interaction between risk markers at baseline in the placebo group and 6-month risk markers in the ARB treatment group with renal/CV outcomes. A two-sided p value of 0.05 indicated the nominal level of statistical significance. Analyses were conducted with R 2.10.1 (R Project for Statistical Computing www.r-project.org).

Results

Baseline characteristics between the ARB and placebo groups in the RENAAL trial was were well balanced.[9] Losartan significantly changed multiple off-target renal or CV risk markers beyond blood pressure. Relative to placebo, losartan decreased UACR, total cholesterol, hemoglobin, and uric acid, it increased potassium, calcium, albumin, and body mass index, while it had no effect on HbA1c and phosphate (figure 1).

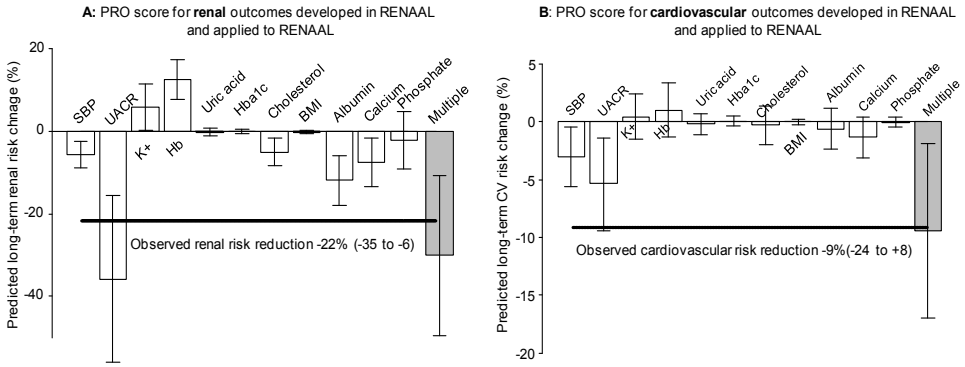
Figure 1: Change in risk markers after 6 months placebo or ARB treatment in the RENAAL and IDNT trials



Estimated renal and CV treatment effect by single and multiple parameter risk response outcome scores

During 3.4 years of follow-up 489 renal and 515 CV events were recorded in the RENAAL trial. Treatment with losartan resulted in a relative renal risk reduction of 21.8% (95% CI, 6.5 to 34.5%; p=0.007) and 9.2% (95% CI -7.9 to 23.6%; p=0.27) relative CV risk reduction, as represented by the horizontal line in figure 2A and 2B, respectively. Single risk markers failed to accurately predict the renal or CV outcome since they either underestimated or overestimated the actual observed drug effect (figure 2A and 2B). The multiple PRO score including all risk markers estimated a long-term relative renal risk reduction of 30.1% (95% CI; 10.8 to 49.5%), which came close to the actual observed relative renal risk reduction (p=0.44), and predicted 9.4% (95% CI 1.9 to 17.0%) relative CV risk reduction which was nearly equal to the observed risk reduction (p=0.98 vs. observed relative risk reduction; figure 2A and 2B).

Figure 2: Observed and predicted long-term relative renal and cardiovascular risk change (%) based on single and multiple PRO scores. Figure A displays the results for renal outcome and Figure B displays the results for cardiovascular outcome. The actual observed treatment effect is indicated by the solid line. The predicted treatment effect based on single and multiple PRO scores are shown by the vertical bars. The PRO score was developed in the RENAAL trial and applied to the baseline and month 6 values of the placebo and losartan treatment arm of the RENAAL trial.



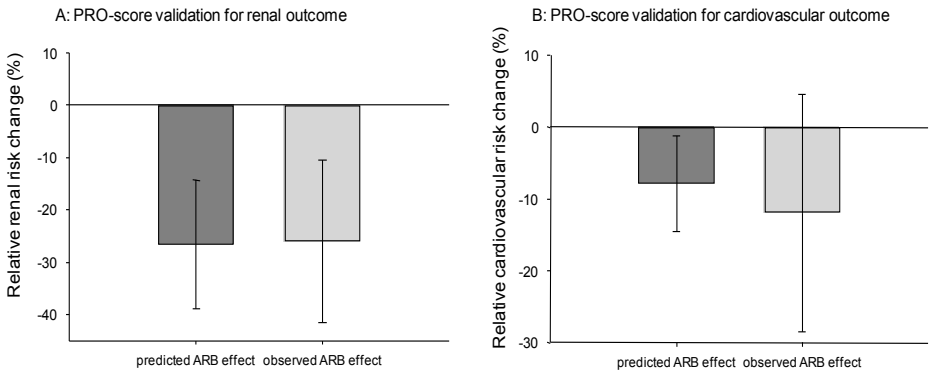
External validation of the multiple parameter risk response outcome score

To test the validity of the PRO score we applied it to an external separate trial database, the IDNT trial, to estimate the treatment effect of the ARB irbesartan on renal and CV outcomes. Irbesartan

caused similar directional changes in renal or CV risk markers as losartan although the magnitude of these changes varied compared with losartan (figure 1).

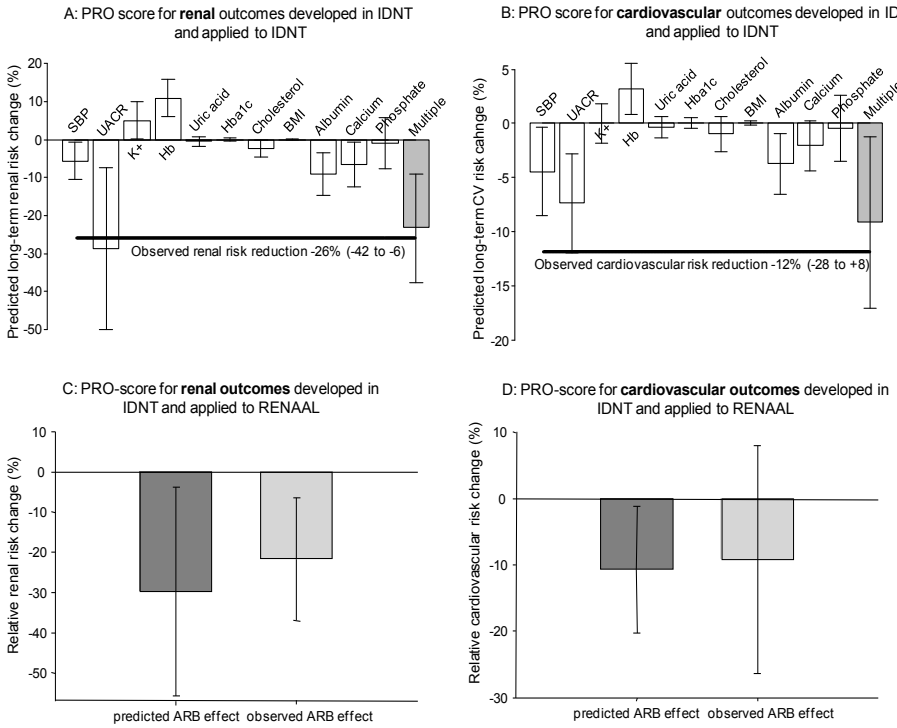
When we entered the irbesartan induced changes in multiple renal or CV risk markers in the PRO score, developed in RENAAL, the PRO score estimated a 26.6% (95% Confidence Interval 14.3 to 38.9%) relative renal risk reduction which was nearly similar to the actual observed relative renal risk reduction of 26.0% (6.4 to 41.5%; p=0.95 vs. predicted drug effect; figure 3A). The PRO score estimated irbesartan's CV treatment effect to be 7.9% (1.3 to 14.5%) which did not differ from the actual observed CV treatment effect of 11.9% (-8.4 to 28.5 %; p=0.67; figure 3B).

Figure 3: Validation of the PRO score in the IDNT trial. Figure A displays the results for renal outcome and Figure B displays the results for cardiovascular outcome. The predicted treatment effect is indicated by the dark grey bar and the actual observed treatment effect is indicated by the light grey bar. The PRO score is developed in the RENAAL trial and applied to the baseline and month 6 measurements of the irbesartan and placebo arm of the IDNT trial.



Development of the PRO-score in the IDNT trial and application to the irbesartan arm of IDNT or losartan arm of the RENAAL trial yielded essentially similar results in that the estimation of the observed treatment effect based on the multiple risk response score outperformed scores based on single risk markers (Supplement figure).

Supplement figure 1: PRO-score development in the IDNT trial and application to the baseline and month 6 measurements of the placebo and irbesartan treatment arm of the IDNT trial (figure A renal outcomes; figure B cardiovascular outcomes). Validation of the PRO score in the RENAAL trial by applying the IDNT developed PRO score to the baseline and month 6 measurements of the placebo and losartan treatment arm of the RENAAL trial (figure C renal outcomes; figure D cardiovascular outcomes



Discussion

We demonstrated that the ARB losartan exerts multiple off-target effects in subjects with type 2 diabetes and nephropathy. These off-target effects are either positively or negatively associated with renal/CV morbidity or mortality. In addition, we showed that only using the short-term change in blood pressure, the on-target effect of this antihypertensive agent, cannot capture the ultimate effect of losartan on renal/CV morbidity or mortality. In contrast, the PRO score, based on short-term drug responses of all (available) on-target and multiple off-target risk parameters, is accurate in predicting the ultimate long-term drug effect on renal or CV outcomes and performs significantly

better than any model based on single on-target or off-target parameters, also in external datasets.

Often drugs change other risk markers than the one they are targeted to, so called off-target effects.We demonstrated that the antihypertensive agent losartan does not only lower blood pressure, but also reduces urinary albumin excretion, hemoglobin, uric acid, and cholesterol, and increases serum potassium, calcium, and albumin. Each of these off-target risk markers are associated with renal or CV morbidity or mortality; [5, 10-12] either they decrease or increase the risk of these events. It is therefore not surprising that a combination of changes in on-target and off-target multiple risk markers more accurately captures the long-term drug effect than changes in single on-target or off-target risk markers.

The blood pressure lowering effect of losartan markedly underestimated the renal/CV protective effects of these drugs despite the fact that these drugs are developed and registered as antihypertensive drugs. Recent trials have shown that this phenomenon is not limited to ARBs but is applicable to other antihypertensive drugs or drug-combinations, [13-16] and even extends to other drugs used in CV risk management as well [17-19] as reviewed in the accompanying article. [20] Taken together, these trials exemplify that the magnitude of renal or CV protection conferred with antihypertensive agents, or other drugs used to mitigate CV risk, cannot always be determined from their on-target drug effect but depends on their composite effect on all on-target and off-target risk markers.

The PRO score uses multiple risk parameters as is done in many other risk estimation engines like the Framingham, UKPDS, or more recent ADVANCE risk engine. What is the advantage of the PRO score? Traditional risk engines in patients with diabetes such as the UKPDS or the more recent ADVANCE risk engine only include traditional CV risk factors and are based on a minimal number of readily available clinical lab parameters to predict individual prognosis. [21, 22] The PRO score is based on many other parameters that are influenced by drug therapy and determine the outcome of the individual, thus increasing the accuracy of the estimates. Indeed, models based on blood pressure, Hba1c and cholesterol alone only predicted ~10% renal and ~2% relative CV risk reductions in the RENAAL trial. In addition, the non-modifiable risk factors age and gender account for a major part of future risk in traditional risk engines and obscure the value of modifiable risk factors. The PRO-score does not include age and gender as they are non-modifiable. By not including age and gender a straighter risk relationship between modifiable risk markers and outcome is obtained which, in case of drug induced changes, offers a better estimate of long-term drug efficacy.

The implications of the present study are multiple. Firstly, a multiple on-target and off-target

PRO score enables more accurate drug efficacy assessment on renal/CV morbidity and mortality by evaluating the short term effect of the drug on a prefixed set of risk markers. This will then help to determine which drugs have the potential to reach the market in early stages of drug development, even before long-term trials are conducted. Secondly, in current registration practices, drugs are registered based on the target of interest. The on-target effects are well measured, recorded and evaluated, whereas the off-target drug effects are usually measured as safety parameters in trials, meaning less rigorous measurement and monitoring. A multiple PRO score including on-target and off-target drug effects may have the advantage to enable more accurate drug efficacy assessment before post-marketing long-term trials are conducted. Finally, the PRO score may offer the physician and the patient a better indication on the prescribed drug effect on long-term outcomes. This makes the PRO-score particularly relevant for the patient-clinician dialogue and will aid to guide the intensity of renal or CV protective drug therapy.

With respect to individual patient care, it should be noted that drug responses within an individual may be different.[23, 24] It has always been assumed that the change in the off-target parameter parallels the response in the on-target parameter within an individual. However recent studies challenge this dogma. For example, a patient may have a reduction in albuminuria but simultaneously experience an increase in blood pressure after initiation of ARB therapy. This implies that the composite effect of a drug on multiple parameters in individual patients should be established and optimized to improve the ultimate drug effect on clinical outcomes.

Several aspects of the model should be considered. As with many prediction analyses, the model depends on the risk markers measured in the trials. A PRO-score consisting of multiple laboratory parameters accurately predicted long-term renal and CV drug effects. Nevertheless, we cannot exclude that two additional risk markers that offset each other were not incorporated in the model. Secondly, the underlying assumption of the model is that the relation between a risk marker and renal or CV outcome is not modified by drug treatment. In other words, the relation between a risk marker and outcome in the placebo group is similar to the relation between a risk marker and outcome after 6 months ARB treatment. We verified the correctness of this assumption in our analyses. Finally, the accuracy of the PRO score depends of the background database such as its size, event rate, its accuracy, and the variation in the levels of multiple risk markers. The current analyses were derived from the placebo arm of the RENAAL trial. This can be improved by increasing the placebo treated background database. The PRO score is only applied to ARB treatment in patients with diabetes and nephropathy. No inferences can be made about the performance of the score to predict long-term effects of other drugs in other disease areas.

The PRO-score is generated from landmark clinical trials conducted in patients with diabetes and nephrology. To base the score on individual patient data from these trials is helpful as the cohort is large and has good follow-up increasing the precision of risk estimates. Furthermore, the trials were conducted in various countries increasing international representation. Some limitations should be addressed as well. The current analysis is a retrospective analysis and requires confirmation in a prospective study. The trials were not sufficiently powered to detect statistically significant treatment effects on CV outcomes compromising the precision of the observed and predicted CV treatment effects.

In conclusion, measuring only the short-term blood pressure effect of ARB treatment, the on-target parameter, may result in misinterpretations in estimating the long-term renal and CV protective effect. This can have major impact on society, individual patients, and drug registration. The PRO score based on multiple on-target and off-target risk markers is accurate in predicting the long-term outcome of ARB in patients with diabetes and nephropathy.

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CHAPTER 6

Summary and future perspectives

Patients with diabetes and nephropathy are prone to develop renal or cardiovascular complications. (1, 2) Given the increasing prevalence of diabetes in combination with the relentless course of the disease new drugs are needed to mitigate the burden of this disease. Unfortunately, despite increased investments in drug development, increased knowledge, and improved methods to discover new drug targets, no new drugs have become available over the last decade that reduce the risk of renal complications. Although attempts have been made to discover novel drugs, and a number of them have indeed been tested in the clinical setting, it appears that during late stage drug development we fail to prove them efficacious. Indeed, recent data show that in diabetes 13% of drugs do not reach the market because of inefficacy or safety issues during late stage drug development.(3) Apparently, we are not very successful to correctly estimate drug efficacy in early stages of development.

The drug development (and registration) process in renal disease management involves the estimation of long-term efficacy and safety. During drug development, the effect of the drug on the risk factor the drug is developed for (such as blood pressure for an antihypertensive drug or HbA1c for an oral glucose lowering drug) is used to estimate the drug's efficacy and to calculate the expected long-term renal protection. The drug effect on these renal risk factors is established during short-term studies, generally up to 6 months of duration. To confirm the estimated long-term efficacy and safety, the short-term drug evaluation is followed by a post-marketing hard-outcome study looking at clinical meaningful outcomes. The latter thus requires large randomized controlled clinical trials.

Recently, numerous drugs have failed to afford renoprotection or appeared to be even harmful despite the drug was beneficial on the target (risk factor) it was developed for. Thus, the drug was not delivering what it was thought to do. For example, avosentan reduced blood pressure (and proteinuria) but increased the risk of cardiovascular events, especially congestive heart failure. (4) Likewise, rosiglitazone was withdrawn from the market because of excess of cardiovascular events despite it lowers HbA1c, the on-target risk factor.(5, 6) These examples unambiguously illustrate the failure to use the short-term effect of a drug on the on-target risk factor to estimate the effect of the drug on hard clinical meaningful renal outcomes and highlight the inability to correctly predict drug efficacy.

Also in patients with diabetes and nephropathy, the reduction in blood pressure induced by Angiotensin Receptor Blockers (registered as anti-hypertensive drugs and the mainstay of therapy in these patients next to blood glucose lowering) does not explain the long-term renoprotective effect. The Reduction of Endpoints in non-insulin-dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy Trial (IDNT) have

shown that losartan and irbesartan respectively delay the progression of renal disease in patients with diabetes and nephropathy.(7, 8) Although both losartan and irbesartan are registered as antihypertensive drugs, the renoprotective effect of these agents appears to be independent of blood pressure. This indicates that these drugs have other effects, so called off-target effects, which contribute to the renoprotective effects of these drugs.

These observations form the basis of the studies conducted in this thesis. In this thesis we investigated which off-target effects ARBs exert and the relationship between these off-target effects with hard renal outcomes in patients with type 2 diabetes and nephropathy. The ultimate aim of the thesis was to combine the on-target and different off-target effects of a single drug into a so called multiple parameter risk response score in order to accurately predict drug efficacy on hard renal outcomes after short-term (6 months) drug treatment.

Off-target effects of various drugs in patients with diabetes

Throughout the thesis we explored the effects of a drug on off-target risk markers. **Chapter 2** provides an overview of the effects of various drugs on novel risk/biomarkers and the relationship between (drug induced) off-target changes in risk/biomarkers and long-term renal and cardiovascular outcome in patients with diabetes. In this chapter we showed that the short-term reduction in albuminuria, transforming growth factor-beta, and N-terminal pro-B–type natriuretic peptide (NT-proBNP) induced by inhibitors of the RAAS were associated with a risk change in renal and cardiovascular outcomes. These data indicate that RAAS inhibition with ACEi or ARB exerts multiple effects on different renal and cardiovascular risk markers which subsequently can change the risk of renal or cardiovascular events.

Off-target effects of ARBs and their relationship with renal outcome

Uric Acid

Serum uric acid (SUA) has emerged as a renal risk marker. Increased SUA has been shown to predict the risk of hypertension, diabetes mellitus, and cardiovascular disease.(9-11) Previous studies have shown that losartan lowers SUA. This hypouricemic effect appears to be largely mediated through reductions in the level of human urate transporter 1 (URAT1) and decreased net urate reabsorption in the proximal tubule.(12) In **chapter 3** we investigated whether losartan exerts off-target effects on uric acid in patients with type 2 diabetes and nephropathy and whether the reduction in uric acid is associated with renoprotection. Indeed, losartan treatment attenuated the rise in SUA levels compared with placebo. The reduction in SUA appeared to be associated with

renal events so that per 0.5 mg/dL decrement in SUA during the first 6 months the risk for doubling of serum creatinine or ESRD reduced by 6% (95%CI 10-3) This association was statistically significant, even after adjustment for a broad range of known renal risk factors. Interestingly, the effect of losartan on SUA explained approximately 20% of its overall renoprotective effect. These data indicate that the off-target effect of losartan on SUA contributes to its renoprotective effect and suggest that SUA is a modifiable risk marker for renal disease, at least in patients with diabetes and nephropathy.

Serum potassium

Although ARBs have several beneficial effects in patients with diabetes and nephropathy, such as decreasing systolic blood pressure, albuminuria, and serum uric acid,(13, 14) they also have other effects that may be related to increased renal risk Treatment with Angiotensin Receptor Blockers is associated with a rise in serum potassium, secondary to decreased aldosterone secretion and impaired potassium excretion. These effects are particularly pronounced in patients with diabetes and nephropathy. In **chapter 4**[a] we confirm that treatment with losartan increased serum potassium concentration, which was in turn associated with an increased risk of renal outcomes and counteract the renoprotective effects of losartan. The increase in renal risk appeared to be independent of other important renal risk factors, such as blood pressure, eGFR, and albuminuria. Thus, although losartan has clearly been shown to be a renoprotective drug, under this protection a renal damaging effect is hiding in those individuals in whom losartan induces high serum potassium levels.

The data reported in chapter 4a indicate that in order to predict the effect of a drug on hard outcomes by using changes in risk markers one should not only evaluate the risk markers that are associated with renoprotection but also take into account the markers that are adversely associated with hard renal outcomes. Currently in drug development, the change in potassium is registered as a safety issue and not as an efficacy issue. However, since the ARB induced change is serum potassium affects its renoprotective efficacy, the change in serum potassium should also be included in efficacy determination and not only separately analyzed as a safety issue. In response to a letter to the editor we provided additional data in **chapter 4**[b] demonstrating that the relationship between increased serum potassium and renal outcome was independent of the severity of the underlying renal disease. Additionally, in this chapter we provided detailed insight in the different patterns of response in albuminuria and serum potassium in individual (sub-group of) patients and their relationship with renal outcomes. It appeared that the response in albuminuria and serum potassium to ARB-therapy was discordant within an individual in a large proportion of patients. This indicates

that within a patient could not response in terms of albuminuria (no reduction) but had an increase in serum potassium, or vice versa, responded in terms of albuminuria (thus had a decrease in albuminuria) but no response in serum potassium. The group of patients in whom both albuminuria and serum potassium rose, and thus had high residual albuminuria and high serum potassium levels during losartan therapy, had a very high risk of renal events. This warrants strategies that optimize the effect of a drug on the good surrogates, such as blood pressure and albuminuria, and to minimize its effect on the bad surrogates, such as serum potassium, in individual patients. This approach may attenuate the high renal risk of the growing population of patients with diabetes and nephropathy.

Integrating the on-target and off-target effects of ARBs to better predict the renoprotective effects

In the chapters 2 to 4b we have shown that ARBs have many other effects than blood pressure lowering alone. The question we addressed in **chapter 5** was whether we could use these off-target drug effects to better estimate the ultimate drug effect of an ARB on hard renal outcomes. We therefore set out to construct a multiple parameter risk response score that incorporates the on-target and off-target effects of ARBs. In this chapter we showed that a multiple parameter risk response score based on 6-month changes in on-target and off-target risk markers can be used to accurately predict the long-term effect of an ARB on hard renal and CV outcomes and performs better than any score based on changes in single risk markers.

Conclusion and future perspectives

In this thesis, the multiple effects of ARBs on various renal risk factors were investigated and the relationship between changes in these risk factors following ARB therapy on hard renal outcomes were analyzed. We have shown that off-target changes in risk parameters contribute to the drug's efficacy either in a positive (enhancing the ultimate drug effect) or negative (blunting of the ultimate drug effect) way. An integrated multiple parameter risk response outcome score, including the on-target and off-target drug effects, was very accurate in predicting drug efficacy on hard renal outcomes. This indicates that an integrative approach, establishing the drug effect on all renal risk factors, is warranted to assess drug efficacy in the absence of hard outcome data. This conclusion has important implications for drug development, drug registration, and individual patient care.

1. From a drug development perspective, one should not only focus on the on-target risk factor but also consider the effect of a drug on multiple off-target parameters as drug induced changes in

these parameters may enhance or blunt the ultimate drug effect. Early accurate assessment of drug efficacy by using the integrated drug effect on all renal risk markers will enable the drug developer to establish which drug has the potential to reach the market in early stages of development and should be further developed as well as it likely prevents lack of post marketing efficacy or safety on hard outcomes. Therefore, a better efficacy estimation of long-term drug effects in early phases of drug development will result in lower drug-attrition rates, less patient exposure to ineffective drugs, and tremendous cost savings.

2. From the regulatory perspective, improved estimation of long-term drug effect will aid to well inform regulatory decisions about novel drugs. In current registration practices, drugs are registered based on the target of interest for which one marker is tested and on being a safe drug for which multiple off-target markers are tested. The off-target drug effects are usually separated from the safety (or off-target) discussion. However, we advocate to change our thinking of drugs in terms of efficacy and safety, but much more about effects that are wanted and unwanted and that all effects contribute to the ultimate outcome. We advocate to classify single drugs that are aimed at long term renal/CV protection as such: renal/CV protective drugs instead of considering them as antihypertensive or oral glucose lowering drugs which implies that they only decrease blood pressure or glucose. This would require an integrated algorithm involving all relevant renal/CV risk markers: the proposed integrated multiple parameter risk response outcome score which likely aids to better drug efficacy assessment and well informed regulatory decisions on novel drugs.

3. From the patient-clinician point of view, using an integrated score of on-target and off-target parameters in clinical practice to determine long-term drug effects in an individual patient score may offer the physician and the patient a better indication on the prescribed drug effect and will aid to guide the intensity of renal or CV protective drug therapy.

The analyses we have conducted are based on already finished trials. Therefore, our findings need to be replicated in ongoing trials to confirm the validity and predictability performance of our multiple risk parameters modeling approach. Validation studies are currently ongoing. If our results can be confirmed in prospective studies, they may contribute to a shift in our thinking how drugs should be developed and monitored in clinical practice.

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Samenvatting en toekomstperspectief

Patiënten met diabetes lopen een hoog risico op het ontwikkelen van nierziekte. In het licht van de toegenomen prevalentie en daarmee samenhangend toegenomen prevalentie van nierziekte zijn er nieuwe geneesmiddelen en nieuwe behandelstrategieën noodzakelijk om de prognose van patiënten met diabetische nefropathie te verbeteren. Echter, ondanks onze toegenomen kennis omtrent de pathofysiologie van diabetes en verworven inzichten in het ontwikkelen van geneesmiddelen zijn er de laatste jaren geen nieuwe geneesmiddelen voor deze patiëntengroep ontwikkeld. Helaas blijkt nog steeds dat ongeveer 13% van alle geneesmiddelen die het eindstadium van het geneesmiddelontwikkelingsproces halen uiteindelijk niet op de markt komt. De meest voorkomende oorzaak is dat het geneesmiddel niet effectief is. Blijkbaar zijn wij dus niet in staat om op juiste wijze de effectiviteit van een geneesmiddel in een vroeg stadium van ontwikkeling in te schatten.

Het geneesmiddelontwikkelingsproces (en geneesmiddelregistratieproces) beoordeelt de effectiviteit en veiligheid van het te ontwikkelen geneesmiddel op basis van risicofactoren voor nierziekte en hart- en vaatziekte. Tijdens de ontwikkelingsfase wordt de effectiviteit van een geneesmiddel beoordeeld op het effect op de risicofactor waarop het geneesmiddel is ontwikkeld (zoals bloeddruk voor een bloeddrukverlagend geneesmiddel en cholesterol voor een statine). Dit effect op de zogenaamde on-target risicofactor wordt gebruikt om uiteindelijk een inschatting te maken van het effect van het geneesmiddel op klinische relevante eindpunten.

In de laatste jaren is er van verschillende geneesmiddelen geen toegevoegde waarde aangetoond bovenop het huidige arsenaal van geneesmiddelen ondanks dat deze nieuwe middelen gunstige effecten hebben vertoond op risicofactoren voor nierziekte. Dit houdt in dat het geneesmiddel uiteindelijk niet het veronderstelde effect opleverde wat gedacht werd op basis van de effecten van het geneesmiddel op de risicofactor. Zo werd bijvoorbeeld avosentan (een geneesmiddel dat bloeddruk en proteinurie verlaagt) in verband gebracht met een hoger risico op hartfalen en werd rosiglitazone van de markt gehaald vanwege het induceren van hartfalen ondanks dat het HbA1c verlaagde. Deze voorbeelden laten zien dat het huidige geneesmiddelontwikkelingst raject, dat gebaseerd is op het inschatten van de effectiviteit van een geneesmiddel gebaseerd op het geneesmiddeleffect op slechts één risicoparameter, tekortschiet.

Geneesmiddelen die een belangrijke bijdrage vormen in het verlagen van eindstadium nierziekte in patiënten met diabetisch en nierziekte zijn de bloeddrukverlagende angiotensine II antagonisten. Uit verschillende studies blijkt echter dat de mate van bloeddrukverlaging geïnduceerd door deze geneesmiddelen slechts in geringe mate de effectiviteit van deze geneesmiddelen verklaart. In de RENAAL en IDNT studies werd aangetoond dat losartan en irbesartan, respectievelijk, het risico op het onwikkelen van dialyse en niertransplantatie verlaagden

ten opzichte van placebo behandeling. Hoewel zowel losartan als irbesartan geregistreerd zijn als antihypertensiva blijkt dat de mate van bloeddruk verlaging in deze studies slechts een zeer geringe bijdrage vormden voor de mate van nierbescherming. Dit suggereert dat geneesmiddelen blijkbaar andere effecten vertonen (off-target effecten) die een bijdrage leveren aan de mate van risicio-bescherming op harde klinische eindpunten.

Deze observaties vormden de basis voor het onderzoek dat beschreven is in dit proefschrift. In dit proefschrift worden de verschillende 'off-target' effecten van angiotensin II antagonisten onderzocht en de relatie met nierziekte beschreven. Het uiteindelijke doel van dit proefschrift is om een risico score te ontwikkelen die zowel de on-target als off-target effecten van een geneesmiddel omvat. Dit als doel om een betere inschatting te maken van de effectiviteit van een geneesmiddel op harde klinische eindpunten.

Off-target geneesmiddeleffecten in patienten met diabetes

In hoofdstuk 2 wordt een overzicht gegeven van verschillende geneesmiddelen die gebruikt worden in de behandeling van type 2 diabetes. In dit hoofdstuk worden de verschillende off-target effecten van deze middelen beschreven en hoe zij in relatie staan met klinische eindpunten. Zo is bijvoorbeeld het NT-proBNP verlagende effect van geneesmiddelen die het renine-angiotensine-aldosterone-systeem remmen geassocieerd met een verminderd risico op nierziekte en hart- en vaatziekte.

Off-target effecten van Angiotensine-II-antagonisten en relatie met nierziekte

Urinezuur

Urinezuur wordt in toenemende mate gezien als een belangrijke risicofactor in de ontwikkeling en progressie van nierziekte. Voorgaand onderzoek heeft aangetoond dat losartan urinezuur verlaagt. Het effect van losartan op de URAT transporter blijkt hierbij een belangrijke rol te spelen. Of de verlaging van urinezuur door losartan ook een bijdrage levert aan het nierbeschermende effect van losartan was onbekend. Dit werd in hoofdstuk 3 onderzocht. Inderdaad bleek dat een verlaging van 0.5 mg/dL in urinezuur door losartan geassocieerd is met een 6% risicoverlaging op het ontwikkelen van eindstadium nierziekte. Deze relatie was statistisch significant en onafhankelijk van andere baseline risicofactoren of veranderingen in risicofactoren.

Kalium

Hoewel angiotensine-II-antagonisten gunstige effecten uitoefenen in patiënten met diabetes hebben zij echter ook effecten die het risico op nierziekte kunnen verhogen. Het hyperkalemisch effect van deze middelen zou het risico op nierziekte kunnen verhogen. Het risico op hyperkalemie geïnduceerd door een angiotensine-II-antagonist is juist verhoogd in patiënten met diabetes en nierziekte. In hoofdstuk 4 bevestigden wij dat losartan kalium spiegels verhoogt. Deze verhoging in kalium was vervolgens onafhankelijk geassocieerd met een verhoogd risico op eindstadium nierziekte. Interessant is de bevinding dat correctie van het behandeleffect van losartan op nierfalen voor het effect van losartan op kalium het beschermende effect van losartan met maar liefst 40% laat toenemen! Dit suggereert dat een deel van de effectiviteit van Angiotensine-II-antagonisten te niet wordt gedaan door het effect van deze geneesmiddelen op kalium. Dus, hoewel het onomstotelijk is vastgesteld dat losartan een nierbeschermende werking uitoefent blijkt dat onder deze bescherming een schadelijk effect schuil gaat dat de effectiviteit van losartan deels teniet doet.

Sommigen hebben zich afgevraagd of de relatie tussen een verhoogde kalium spiegel en eindstadium nierziekte niet het gevolg is van onderliggend nierschade. Naar aanleiding van een 'letter to the editor' hebben wij in hoofdstuk 4b extra analyses uitgevoerd om aan te tonen dat de relatie tussen kalium en eindstadium nierziekte niet wordt bepaald door onderliggend nierschade.

Integratie van on-target en off-target geneesmiddeleffecten om de nierbeschermende effecten van angiotensine-II-antagonisten beter te voorspellen

In hoofdstuk 2 tot en met 4 hebben wij verschillende off-target effecten van Angiotensine-II-antagonisten beschreven. In hoofdstuk 5 integreerden wij deze effecten in één risico score om een betere inschatting te maken van geneesmiddeleffectiviteit. We laten in dit hoofdstuk zien dat een score die zowel de on-target als de off-target effecten van een geneesmiddel bevat de beste schatting oplevert omtrent de effectiviteit van een geneesmiddel in het voorkomen van nierziekte en hart- en vaatziekte.

Conclusie en toekomstperspectief

In dit proefschrift is aangetoond dat off-target geneesmiddeleffecten van angiotensine-II-antagonisten een belangrijke bijdrage kunnen leveren in de effectiviteit (zowel in positieve als in negatieve zin) van een geneesmiddel in het voorkomen van nierziekte. De integratie van alle off-target effecten met het on-target geneesmiddeleffect levert uiteindelijk de beste voorspelling op omtrent de effectiviteit van een angiotensine-II-antagonist op klinische relevante eindpunten.

Deze bevinding heeft belangrijke consequenties voor zowel geneesmiddelontwikkeling, geneesmiddelregistratie en individuele patiëntenzorg.

1. Wat betreft geneesmiddelontwikkeling is het van groot belang om in een vroeg stadium inzicht te krijgen in de effectiviteit van het geneesmiddel op klinische eindpunten. Op basis van deze inzichten kunnen besluiten worden genomen om al dan niet het ontwikkelingsproces door te zetten en kunnen grootschalige geneesmiddeltrials op een efficiënte manier worden opgezet.
2. Vanuit het oogpunt van geneesmiddelregulering zal een betere voorspelling van het geneesmiddeleffect op klinische relevante eindpunten de regelgevinginstantie kunnen helpen om juiste beslissingen te nemen of nieuwe geneesmiddelen op de markt kunnen worden toegelaten. Op dit moment worden off-target geneesmiddeleffecten niet meegewogen in de discussie omtrent geneesmiddeleffectiviteit maar worden zij apart beoordeeld als veiligheidsparameters (denk hierbij bijvoorbeeld aan kalium). Wij zijn voorstander om alle off-target effecten mee te nemen in de beoordeling van geneesmiddeleffectiviteit en in dit opzicht zo weinig mogelijk scheiding aan te brengen tussen geneesmiddeleffectiviteit en veiligheid. Om de effectiviteit van een geneesmiddel op basis van risico factoren te beoordelen is een geïntegreerd algoritme noodzakelijk, de door ons ontwikkelde score kan in dit opzicht gebruikt worden.
3. Tot slot kan vanuit het perspectief van individuele patiëntenzorg een geïntegreerde score zowel de patiënt als de behandelaar een beter perspectief geven omtrent de effectiviteit van een geneesmiddel en zou van dienst kunnen zijn in het optimaliseren van de behandeling voor de individuele patiënt.

De resultaten besproken in dit hoofdstuk zijn verworven uit inmiddels afgeronde trials. De resultaten (met name de on-target / off-target risico score) moeten nu bevestigd en gevalideerd worden in geneesmiddeltrials die op dit moment lopende zijn. Indien de resultaten besproken in dit proefschrift gerepliceerd en gevalideerd worden kan dit een belangrijke verandering te weeg brengen in de manier waarop geneesmiddelen worden ontwikkeld en beoordeeld; namelijk niet richten op een enkele risicofactor maar op basis van een geïntegreerd algoritme dat alle effecten van een geneesmiddel op risicofactoren integreert.

CHAPTER 7

Dankwoord

After four years time staying in the Netherlands, I am going to finish my PhD study. During this period, I believe that I learned many things which can substantially help my future career, and many unforgettable moments I think a person worths to experience from life experience point of view.

I sincerely thank my promotors, supervisor, professors and colleagues from Groningen and Utrecht for their great support, valuable ideas and suggestions in all the meetings and discussions. For my friends in life, no matter wherever you are, thank you for the time we spent together. And my thanks for my family in China for their support and love.